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THE JOURNAL OF EXPERIMENTAL MEDICINE

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THE JOURNAL OF EXPERIMENTAL MEDICINE

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The present number of the JOURNAL OF EXPERIMENTAL MEDICINE, which has been edited by the undersigned, completes Volume Sixth. Articles which have accumulated during the period of suspended publication now appear, in most instances, from one to two years after their completion. Hereafter the JOURNAL will be published under the auspices of the Rockefeller Institute for Medical Research and under new editorial management. It will, as heretofore, be devoted to the publication of original investigations in the domain of the medical sciences and will, at the same time, serve as a medium of publication for the institution by which it will be issued. With the support of those who have formerly contributed and subscribed to the JOURNAL, the attempt will be made to maintain, uninterruptedly, the standard established by its first editor.

SIMON FLEXNER,
EUGENE L. OPIE.

THE PRODUCTION OF SARCOSPORIDIOSIS IN THE MOUSE BY FEEDING INFECTED MUSCULAR TISSUE.

By THEOBALD SMITH, M. D.,

George Fabyan Professor of Comparative Pathology, Harvard University.

Plates I–IV.

Since the discovery of sporozoan parasites belonging to the order now known as Sarcosporidia in the muscle fibres of the house mouse by Miescher in 1843, many other mammals and not a few birds have been found to harbor them. Some species, like the one described by Miescher, are quite conspicuous to the naked eye in the ultimate stage of development. Certain others, including those of swine, are microscopic. Those of cattle may be seen as barely visible, whitish specks when situated beneath the endocardium. Some of the species found in birds are fairly conspicuous as shown by the dimensions given by Stiles.[1]

The record of sarcosporidiosis in man is meagre and in part uncertain in value. Only two cases—one described by Kartulis[2] and one by Baraban and Saint-Remy[3]—seem to be genuine. In those of Lindemann[4] and Rosenberg[5] the diagnosis must remain doubtful. The case of Kartulis was that of a Sudanese. An abscess had formed in the liver and had extended to the abdominal wall. The sarcosporidia cysts were found in considerable numbers both in the liver and

[1] On the presence of Sarcosporidia in birds. Bulletin No. 3, p. 79, *Bureau of Animal Industry*, Washington, 1893.

[2] *Zeitschr. f. Hygiene*, 1893, xiii, p. 1.

[3] *Bibliographie anatomique*, 1894, p. 79; *Comptes rend. Soc. de biol.*, 1894, 10. s., i, p. 201.

[4] From the Russian in *Deutsche Zeitschr. f. Staatsarzneikunde*, 1868, n. F., xxvi, p. 326.

[5] *Zeitschr. f. Hygiene*, 1892, xi, p. 435.

the muscular tissue forming the periphery of the abscess.[6] Baraban and Saint-Remy found genuine sarcosporidia cysts in the muscular fibres of the vocal cords of an executed criminal. The cysts, from .077 mm. to .168 mm. in diameter, were chambered and each chamber was filled with falciform bodies or sporozoites. These two cases indicate that some one or more of the muscle parasites of the lower animals may reach maturity in the human subject.

This brief epitome of sarcosporidiosis in man leaves to this subject but little that is of immediate practical importance to human pathology. It should not be forgotten, however, that the muscular system in man is not subjected to that scrutiny which the viscera undergo in pathological enquiries and that Sarcosporidia may be present and yet not be recognized. As in trichinosis, the invasion may be overlooked or the attending symptoms misinterpreted and the subsequent quiescent stage fail to arouse any attention. The fact that the etiology of acute primary polymyositis is still unsettled should lead to more careful examination of the muscular system as a part of routine pathological work. It is not improbable that invasion of the muscular system by aberrant parasites may take place now and then. Such parasites being in the wrong host may fail to develop and hence elude detection, while the products resulting from their disintegration may lead to inflammatory reaction.

The supposition that Sarcosporidia are transmitted by way of the digestive tract has been a favorite hypothesis of investigators and has been frequently tested by feeding infected muscle. No positive results have been reported. The failures were, no doubt, in part at least, due to the method of experimentation. The endeavor to transmit Sarcosporidia of sheep by injecting them into white mice and guinea-pigs as was done by Kasparek,[7] or by the eating of raw meat by human beings as was done by Moulé,[8] seems at best abortive, since

[6] Max Braun in reviewing this case did not hesitate to regard the diagnosis of Sarcosporidiosis as extremely doubtful, but he subsequently fully agreed with Kartulis after having examined the latter's preparations (*Centralbl. f. Bakteriol.*, 1895, xviii, p. 13).

[7] *Centralbl. f. Bakter.*, 1895, xviii, p. 327.

[8] Des sarcosporidies et de leur fréquence, principalement chez les animaux de boucherie. Paris, 1887.

these parasites are highly specialized forms. It is a priori not improbable that the same species of Sarcosporidia may infect several hosts possessing certain, to us unknown, relationships, but to gain an insight into the life history of such forms the problem first to be solved is to determine the mode of transmission in the same species in which they naturally occur.

The negative results of recorded transmission experiments, to which we may safely add many unpublished ones, induced me to make an effort to fill at least a portion of the existing gap when, in 1898, I found a large proportion of gray mice which had been kept in a large wooden cage for over a year infected with Sarcosporidia. The large number of infections suggested that transmission occurred directly and not through an intermediate host. The occasional discovery of dead mice in this cage, of which parts had been consumed, as well as the not infrequent mutilation of the tails and scrota of living mice, led to the hypothesis referred to above that the infection takes place through the digestive tract in a manner analogous to the transmission of Trichinæ. Though the experiments needed to demonstrate the truth or falsity of this hypothesis seemed at first simple and easily executed, yet in the course of carrying them out a number of factors appeared which had to be taken into account. The length of time consumed by each feeding experiment, about 3 months, permitted the introduction of the new control factors only slowly, so that these tests have extended over a period of more than 2 years.

In order to present the more important facts of these investigations in an intelligible manner, I shall first relate the life history of the muscle parasite as interpreted by the results obtained. I shall then give such details of the actual experiments as may be necessary to enable others to estimate their relative value in establishing the results claimed and to repeat them if thought desirable.

Sarcocystis muris (R. Blanchard) Labbé.[2]

(*Miescheria muris*, R. Blanchard, 1885.)

The parasite, as a rule, is noticed only in its matured state when it becomes visible to the naked eye. It seems to be pretty widely dis-

[2] *Das Tierreich. Sporozoa*, p. 119. Berlin, 1899.

seminated and infected mice are encountered at intervals in most laboratories where mice are kept, to judge from verbal accounts of such discoveries in this country. The frequency with which they are found in rooms whither mice are drawn by ample food supply and where they breed is discussed in a subsequent section of this paper.

A mouse whose muscular system contains matured parasites presents a very striking appearance. If the skin be removed all the skeletal muscles will be seen to contain streaks of a whitish color. These may be so numerous as to leave but little of the normal red color of the muscle or they may be quite scarce (Plate IV, Fig. 7). Even the muscles of the head and the diaphragm are invaded. I have found them in sections of the eye muscles. The heart muscle, however, remains free. These linear streaks run parallel to the muscle fibres. In case of superimposed translucent layers whose fibres run in different directions, as in the abdominal muscles, the streaks are seen crossing one another at different angles. They vary more or less in length, the oldest and largest being from 1 to 1.5 cm. long and about .25 mm. broad. The more isolated ones are of greater width than those crowded together.[10]

If a piece of fresh muscle be teased in some indifferent fluid and examined under a low power the whitish streaks resolve themselves into opaque, thin-walled tubes, densely packed with crescentic bodies, the so-called sporozoites.[11] The outer wall of such a tube is very thin, smooth, and without any external appendages. It is usually encased by a narrow rim of muscle fibrillæ, the remains of the invaded fibre. Within this parasitic tube, among the sporozoites, a faint network of lines is discernible which forms flattened meshes whose long axes are at right angles to that of the parasite itself. These are

[10] According to investigations made by Mrs. Gage (*The Microscope*, 1888, viii, p. 225) some of the fibres in the shorter muscles of the mouse extend from tendon to tendon. In the longer muscles they are as a rule shorter than the muscle itself. Exact measurements of isolated fibres showed a variation in length of from 5 mm. in the short muscles to 22 mm. in the longest muscles. I have not made any measurements upon isolated infected fibres, but all observations point to the inference that the parasite eventually occupies the entire length of the muscle fibre in which it happens to lodge.

[11] The terminology used in this article is that suggested by Schaudinn (see Lühe's review in *Centralbl. f. Bakt.*, 1900, xxvii, p. 367).

optical sections of partitions which divide the entire parasite into chambers which enclose the sporozoites (Plate I, Fig. 1). When the parasite is torn by teasing, the latter escape and are found isolated (Plate III, Fig. 5). In outline they are crescentic and resemble bananas. The body in the middle is .004 mm. thick and measures in a straight line from end to end .012 mm. A modicum of fine granules is recognizable but nothing more.

When these sporozoites, suspended in normal salt solution, are warmed to 35° or 37° C. they exhibit peculiar movements. These are better observed in slide cells with convex bottom than in the hanging drop. By melting down the corners of square cover-glasses, as recommended by Siedlecki, good cells may be improvised with ordinary slides. The cells must be sealed with vaseline to prevent the concentration of the salt solution. The observations are most satisfactorily made with the aid of a Nuttall thermostat into which the entire microscope is placed.

It will be noticed as the temperature rises that these bodies have become more homogeneous and somewhat refractive and that their form has become slightly altered. They are now more curved and somewhat more slender. At 34° to 36° C. they begin to move in a gliding manner on a circumference corresponding to their own curvature for from 1½ to 2 revolutions. Then they suddenly flop over, *i. e.*, revolve on their long axis ¼ to ½ a revolution. After from 15 to 30 seconds of rest they pass through the same motions anew. Flagella were not observed. These movements may be observed for several hours. During the second hour they grew less and less vigorous, and finally only a few remained active. They are dependent on warmth, ceasing when the slide is removed from the thermostat or allowed to cool, and beginning again when it is warmed. I have seen a few move in midsummer at a temperature of 29.5° C. In the only experiment made to test the vitality of the sporozoites after the death of the host they retained their power to move for at least 4 days, the mouse tissues being kept in the refrigerator in the meantime and examined from day to day.[12] These observations lead to

[12] In some notes made in 1890 when this parasite came under my observation in a single case (Washington, D. C.) the same peculiar movements are recorded. The

the inference that the sporozoites, owing to their perishable nature, must enter another host, presumably warm-blooded. The movements simulate a boring or screw-like action, which may come into play when they penetrate the mucosa.

When muscular tissue containing the matured sporozoites was fed to mice, no evidence of any invasion of the muscle fibres was obtained until approximately the 45th day, when the smallest parasites were first detected. The youngest parasite which I have recognized in fresh tissue was a fusiform body .152 mm. long and .02 mm. broad (Plate IV, Fig. 8). This stage consists of a delicate, structureless membrane whose contents, in the fresh condition, are hyaline and practically invisible. A moderate number of minute, scattering, refringent granules appear in its substance, which are of substantial aid in finding it. In somewhat later stages when the parasite has grown much longer, its substance is found divided into a number of broadly fusiform bodies whose long axes are nearly parallel to that of the mother tube (Plate II, Fig. 4). In this stage the parasite readily slips out of the muscular fibre in which it is lodged, when the fresh tissue is teased. It suggests a tube densely packed with small fish. These primary divisions of the parasite are .012 mm. long and .004 mm. broad in the middle. Each is provided with a spherical highly refringent granule .001 mm. in diameter blackening slowly in osmic acid. It is contained in a minute vesicle. Beyond this the fusiform bodies reveal no structural details. Individuals are now and then found in which the division, completed in some parts, lags behind in others. The bluntly rounded ends of the parasite are usually behind the rest of the body in this respect.

This primary stage of the fusiform bodies is followed by another, seen first in the central portion of the tube. Here the parasite becomes broader and more opaque owing to the presence of closely packed crescentic or kidney-shaped bodies (Plate IV, Fig. 11). Just what change takes place in the fusiform bodies which leads to the crescents I am unable to state. Certain appearances suggested a

sporozoites of the Sarcosporidium of sheep are described by L. Pfeiffer (*Die Protozoen als Krankheitserreger*, p. 123. Jena, 1891) as becoming amœboid when warmed in human saliva.

longitudinal cleavage of the former. One parasite in this transitional stage I was fortunate enough to obtain, by teasing, completely freed from its encasing fibre. It was about 4 mm. long and .026 mm. broad.

In about 70 days after feeding the parasites enter the stage of spore and sporozoite formation. The substance of the parasite is now found to be made up of relatively large roundish or polyhedral masses of a finely granular appearance which are in close apposition with one another and .014 mm. to .016 mm. in diameter (Plate IV, Fig. 12). These sporoblasts soon break up into the final sporozoites, the exact number of which I have not been able to determine. Probably eight are formed from each sporoblast. The continuous growth and breaking up of the sporoblasts causes enough internal pressure to force the sporocysts to assume a flattened outline within the parent tube, with their long diameter at right angles to that of the latter. The whole suggests the appearance presented by dried figs in their original package. The stage of the unsegmented sporoblast I have rarely seen, and I think it is of short duration, the sporocyst following closely upon this stage. Rarely sporoblasts and sporocysts are found in different sections of the same individual at the same time. As stated above, the ripe sporozoites readily escape from the parasitic tube when fresh tissue is teased. A similar escape is not observed, however, in any of the preceding stages. Neither the fusiform nor the succeeding crescentic bodies leave the parent tube when the latter is torn unless decomposition has set in or a one per cent solution of acetic acid be added.

The account given above implies that the partitions within the Sarcosporidium are not ingrowths from the external wall as is frequently claimed, but that they are simply the walls of the sporocysts in close apposition with one another. I have seen nothing in my observations that would lead me to accept any other view with reference to the species under consideration.

Sarcocystis muris thus ripens within the muscular tissue in 2½ to 3 months after the date of feeding. Ingestion of infected muscle at this final stage by another mouse is followed by a similar infection of the muscular system.

It was not my purpose at this time to enter into any study of the morphological details of the various stages in the intra-muscular life of this Sporozoon. It seemed, however, desirable to confirm the observations made with fresh tissues upon such as were fixed and hardened and stained according to current methods. In doing this the following plan was adopted:

Muscular tissue from chloroformed mice was fixed for 24 hours in Zenker's fluid and then, after thorough washing, was hardened in ascending strengths of alcohol. The tissues were embedded in paraffin after passing through cedar oil and cut in ribbons. Various stains were employed, such as the various hæmatoxylin preparations with or without eosin and picro-acid-fuchsin; also eosin and Unna's polychrome methylene blue, and Heidenhain's iron hæmatoxylin. The best stain I found to be hæmatate of ammonia, which I had prepared myself and which stained rather slowly and chiefly nuclear matter.

In the earliest intra-muscular stages of the parasite seen, a multiple division of the nucleus was already under way (Plate I, Fig. 2, and Plate II, Fig. 3). The entire substance was mapped out into a large number of areas having certain definite characters. Each area consisted of a rather dense nuclear body, .002 mm. in diameter, and with faintly lobulated periphery occupying a clear space 2 to 3 times its diameter. Within this space and a somewhat variable distance from the nucleus was another body, perfectly spherical, about .0005 mm. in diameter and staining compactly with chromatin dyes. The significance of this minute satellite accompanying each nucleus must be left to future cytological studies.[13] All that I can state now is its unvarying presence in this early stage. It has not been described by Bertram,[14] who has studied the early stages of other species of Sarcosporidia.

A division into separate individuals or cells was not noticed in the earliest stages. Somewhat later fusiform bodies appear in outline, each of which contains the nucleus and the micro-nucleus or karyosome above mentioned. The latter, however, soon disappears.

Beyond this the study of stained sections has not thus far contributed anything material to the facts brought out by the study of fresh tissue. No clue concerning the suspected division of the fusiform bodies before they enlarge into the sporoblasts was obtained. Nor did the examination of sections from a number of cases reveal the processes in the

[13] For similar bodies in the development of trypanosomes of gray rats see Rabinowitsch and Kempner, *Zeitschr. f. Hygiene*, 1899, xxx, p. 251.

[14] *Zoolog. Jahrb., Abtheilung f. Anat. u. Ont.*, v.

sporoblasts which end in the breaking up of these into sporozoites. Evidently these changes go on very rapidly and are encountered only through lucky accidents rather than carefully planned and executed experiments.

This fragmentary statement of what seem to be the stages leading to the formation of the sporozoites has been introduced simply as a stimulus to a more thorough study of this parasite, which feeding experiments enable us now to obtain in any desired amount. The statements of other observers point to a similar complex development of other members of this genus. Thus Bertram,[15] in a study of the Sarcosporidia of sheep speaks of the earliest divisions or cells as mother-sporoblasts, implying thereby a further division before the stage of sporoblast is reached. L. Pfeiffer[16] thinks that there may be crescentic bodies of the second, third, etc., generation.

EXPERIMENTAL DATA AND RESULTS.

Since the experiments whose results I have briefly given are the first in which feeding has been successful, it is necessary to go somewhat into detail concerning the experiments themselves in order to point out the difficulties and limitations inherent in work of this kind to which bacteriological methods are inapplicable. The details which require special attention are: (1) the source of the animals used; (2) the food; (3) the association of the animals with one another in confinement: (4) the ecto-parasites of mice; and (5) the method of feeding the infected material.

1. The common gray house mouse was used almost exclusively. A few white mice and crosses were included and the results indicated equal capacity for infection. Since it is impossible to tell, without removing bits of muscular tissue by a surgical operation, whether any mouse is infected spontaneously, even a severe infection being compatible for many months with a sleek appearance and the usual vivacity, control observations were made upon mice during the whole period of the investigation. The surgical operation referred to was

[15] Loc. cit.

[16] *Die Protozoen als Krankheitserreger*, p. 119. Jena, 1891.

considered inapplicable in the case of such small animals, not to mention the chances for missing slight infections. The chief source of the mice was a room where the small laboratory animals were kept. Mice caught in this room as well as elsewhere were examined microscopically. Portions of muscular tissue were teased fresh in normal salt solution and carefully scrutinized with the 16 mm. and 4 mm. objectives for those earlier stages which are not visible to the naked eye. 155 control mice, of which 120 were from the animal room above referred to, were examined in this way. Of the 35 from outside sources, one was found infected. Of the 120 from the animal room examined over a period of 3 years, 8, or about $6\frac{2}{3}\%$, were found infected. This rather high rate of infection is probably due to the fact that before attention had been directed, in the spring of 1898, to the infected cage a mouse may have occasionally escaped from it and carried the infection into the hiding places. A tabulation of all the facts bearing upon these control mice showed that there was, during the winter months of both 1900 and 1901, a slight wave of infection, which, judging from the age of the Sarcosporidia, started near the beginning of December. It was almost wholly confined to the large adults, male and female, and was most probably due to some old mouse killed and partly consumed by others.

2. The association of mice in confinement may lead to general infection if a spontaneously diseased mouse should die in the cage. In order to eliminate this source of error the mice were either transferred directly to glass jars, one or two in a jar, or else when they were stored in cages in larger numbers, any that died or were removed for other purposes were carefully examined.

3. The food of the mice was at first bread and oats, later oats almost exclusively. The possibility that the oats might be contaminated led to steaming them before use. The general outcome of the experiments does not favor the hypothesis that infection may occur in this way. The bedding for the jars was carefully selected from hay and straw, and in the later experiments was sterilized dry as a final precaution.

4. The negative outcome of the feeding experiments of former

observers taken together with the somewhat unexpected results of investigations upon other Protozoa which demonstrated that arachnids are the transmitters of bovine malaria (Texas fever), mosquitoes of human malaria, that flagellates in the blood of rats are transmitted by fleas, in the blood of larger animals by a fly (*Glossina morsitans*), made it necessary to examine any possible relationship between sarcosporidiosis in mice and certain ecto-parasites which infest them. There is, however, no close analogy between the transmission of microorganisms circulating freely in the peripheral capillaries and such parasites as are situated more deeply and securely in the muscle fibres. The ecto-parasites of the mice which came under my observation were two species of mites, *Myobia musculus* and *Myocoptes musculinus*. They were either found on different animals at different times or else associated together on the same individuals. In order to bring them into view, the skin of the chloroformed mouse was left from a few to 24 hours under a bell-glass. Any mites would then appear on the hairs, usually near the tips. *Myocoptes* was first encountered as a delicate woolly scab around the base of the ears. In this colonies of eggs and immature young were found. More rarely colonies of young were found in the subcutis as round, flat masses not more than 1 mm. in diameter and resembling a little bit of circumscribed adipose tissue. Adult *Myocoptes* were occasionally found in large numbers on unthrifty caged mice. As a rule mice confined together in numbers up to 15 or 20 were infested.

The possible relation of these mites to the sarcosporidia was carefully taken into consideration by noting their presence or absence on the animals examined. As they are very small and cannot be made out distinctly without a hand-lens, they may be overlooked when very few are present.[17] They did not seem to bear any etiological relation to the muscle parasite. They were frequently noted as absent in positive cases and very numerous in negative cases. Long cohabitation with infected mice in the presence of these mites did not increase the opportunity for infection, as will be pointed out farther on.

[17] *Myobia* is about .5 mm. long and .2 mm. broad. *Myocoptes* is about .3 mm. long and .13 mm. broad. These dimensions apply only to adults.

5. As stated above, the mice used in the experiments were either kept before the feeding in lots of 10 to 15 in large cages made of wood and fine-meshed wire, or else they were placed directly into glass jars. Ordinary battery jars, 6 by 8 inches, were covered with a galvanized-iron wire top with meshes too small to admit house flies. From this top a small glass dish to hold water was suspended with copper wire. The drinking water was renewed by removing the wire top and slipping a glass plate in its place temporarily. The drinking dish left attached to the wire top was rinsed and filled and the top replaced. This arrangement kept the bedding dry and the jars were changed but once in three weeks. The grain was poured into the jar and the hulls slowly accumulating were removed when the jar was changed. In such jars two, very rarely three, were kept.

In experiments from No. 8 to 19 of the table, the infection of the mice was effected in the following manner: Food was usually withheld for the day, and towards evening the muscular tissue of the infected mouse, freed completely of skin and entrails, was cut up into fine bits in normal salt solution. This mass was mixed with bread crumbs in a small glass dish and moistened if necessary with more salt solution. Each jar, containing one or two mice, received a dish. The amount eaten was noted next morning. In the earlier experiments the muscular tissue or a piece of the carcass was placed in the jar without any preparation, but the mice often refused to touch it. The food substances mixed with the infected flesh must be properly chosen. I have found bread crumbs softened in normal salt solution a favorite dish. In the summer of 1900 I used cornmeal, but the mice threw it out of the dishes, and I attribute the failure of certain experiments to the injudicious selection of the food. The mice to be examined were usually chloroformed, more rarely killed by a blow while under chloroform. The latter did not affect the vitality of the Sarcosporidia or of the mites.

When the preliminary experiments had made it evident that the Sarcosporidia enter the muscles by way of the digestive tract, it became necessary to determine the time after feeding when the parasites begin to appear in the muscle-fibres or can be recognized therein, and the time required for the ripening of the sporozoites. In order to eliminate errors of interpretation it was desirable to examine the animals during the earlier, most characteristic stage of the muscle parasite, because the later ripe stage remains unchanged indefinitely

and gives no clear testimony concerning the probable date of infection. To determine the time of the earliest appearance of the parasite in the muscle fibre, infected mice were killed at different periods after the feeding and the quite uniform answer given by all experiments was that the earliest stages were detected between the 40th and the 50th day after feeding.

The accompanying table has been condensed from one upon which all the details of the experiments to which any attention had been given were noted. Among these are the source, size and sex of each mouse, the date of capture, the mode of confinement and the presence or absence of mites. A study of these data led me to regard most of them as negligible factors, and they are therefore omitted from the published table.

In the examination of the mice, a careful record was kept of the stage of development of the muscle parasite, and upon this record is based the number of cases which are put down as the result of artificial infection and the number in which the infection had presumably occurred in the natural way.

Before discussing the results of the experiments as a whole, a few remarks concerning some of them may not come amiss. One of the earliest (No. 2) led me to assume erroneously that the parasite appears very soon—within a week—in the muscular tissue. Six days after the single mouse had been fed it died, and in the abdominal muscles were found the earliest segmenting stages of the parasite. Later experiments made it evident that the mouse was infected when used. Somewhat later the feeding experiments made with the carcass of infected mice yielded results which seemed to point to wound infection. Those fed with bony portions were found more frequently infected. This inference was also found to be an error and was given up when the feeding of bread with the infected muscle proved so successful. Of the later experiments, No. 12 deserves notice. The 2 mice which were caught in a distant building and did not come in contact with any others were both found in the earliest stage of infection on the 51st day. Experiments No. 13 and 14 yielded very poor results, yet these were offset by the fact that there was no evidence that any mouse had been infected before use. The partial failure to infect I attribute, as already stated, to the use of cornmeal. On several occasions I have noticed that mice recently caught in distant buildings were less inclined

Number of experiment.	Number of mice used.	Source of mice.		Date of feeding.	Manner of feeding.	Mice examined						Number evidently spontaneously infected.	Number infected by feeding.	Source of infected muscle.
						Before 45 days after feeding.		Between 45 & 60 days after feeding.		Later than 60 days after feeding.				
		Animal room.	Elsewhere.			Infected.	Not inf.	Infect.	Not inf.	Infect.	Not inf.			
1	1		1	Nov. 18, 1898	Portion of body of infected mouse.	0	1							
2	2	1	1	Jan. 4, 1899	" " " "	1	1					1		
3	4	?	?	Feb. 1, "	" " " "	0	4							
4	4	?	?	Feb. 24, "	" " " "					1	3		1	
5	2	?	?	May 20, "	" " " "					2	0		2	
6	2	?	?	May 22, "	" " " "					1	1		1	
7	6	?	?	Sept. 15, "	" " " "	0	4			1	1		1	Expt. No. 6.
8a	8	animal room	—	Oct. 3, "	" " " "	0	4	2	0	2	0		4	
8b	6	" "	—	Oct. 3, "	Muscular tissue + bread + salt sol.	1	1	4	0			1	4	Expt. No. 5.
9	11	" " & elsewhere		Dec. 9, "	" " " "	0	7	2	0	1	1	1	2	Expt. No. 8.
10	7	1 (?)	6	Jan. 15, 1900	" " + corn meal + "	0	1	2	1	2	1	1	3	Infected cage.
11	4	3	1	Feb. 22, "	" " " "	1	0	3	0			1	3	" "
12	2		2	Mch. 28, "	" " + bread + "			2	0				2	Expt. No. 10.
13	12	9	3	May 9, "	" " + corn meal + "			4	2	2	4		6	Infected cage.
14	18	18		June 26, "	" " " "	0	6	1	2	2	7		3	" "
15	2		2	Aug. 25, "	" " + bread + "			1	0	0	1		1	Expt. No. 13.
16	10		10	Sept. 19, "	" " " "	0	1			7	2	1	6	" " 14.
17	8	4	4	Nov. 25, "	" " " "	0	1			5	2		5	Infected cage.
18	17	11	6	Dec. 18, "	" " " "	0	9			6	2		6	Expt. No. 16.
19	18		8	Mch. 23, 1901	" " " "			6	2				6	" " 17.
					Totals.....	3	40	27	7	32	25	6	56	

to eat the infected food than those caught in the animal room or confined for some time in cages or jars, where food was always plentiful. Possibly the changed environment leads to a more voracious appetite, which may influence the results of experiments. In not a few instances only one of two mice fed together in the same jar was found infected.

Before leaving the subject of minor details, I shall give an account of Experiments No. 18 and 19, which may be taken as a type of the later ones. In No. 18, 18 mice were used, of which one was found dead in the course of the experiment, but too late to be examined, leaving 17. 11 of these were from 3 cages stocked during the summer of 1900. Control examinations of all that died in the cages were made. Two from a distant house were placed together in a jar. Four were white mice raised in another house and also kept by themselves. On the day of feeding the 11 gray mice had been confined for 3 to 4 months and were not very thrifty. Of these, 9 were chloroformed from 2 to 41 days after feeding, but no infection was detected. The remaining 2 were examined on the 60th and 62d days respectively, and the infection was found in the early stages. The 2 gray mice from a distant house confined only 2 days before feeding refused the infected food. Both were found free from infection on the 94th day. The 4 white mice were examined 67, 69 and 85 days after feeding and were found in stages of infection corresponding to the assumed cycle of development of the parasite. Both negative and positive cases were infested with mites.

In No. 19, all the mice were from a distant house and kept separate from those caught in the animal room. There were 4 jars, containing 2 apiece. Between the 50th and 60th days all were chloroformed. 6 out of 8 were in the earliest stages of sarcosporidiosis. In two jars both mice were affected. In the remaining two, only one each. A fifth pair of mice from the same source, and kept in the same way as controls, were killed at the same time. Both were free from infection.

Turning to the results of these experiments, we find that of 43 mice killed before the 45th day, 3 were infected. On account of the advanced stage of the parasites, these are classed as infected before use. Of the 34 killed between the 45th and the 60th day, 27 were infected. Of these, one is classed with the spontaneous infections. Of the 57 killed after 60 days, 32 were infected. Of these, 2 are rejected as previously infected. Taking all together, we have of 91 examined after 45 days, 59 infected, 32 not. Rejecting 3 of these, we have an infection of 63.6% after 45 days. If we compare the

spontaneous infections discovered in the experimental mice with those of the mice examined as controls, we find a close agreement. Eliminating from both series all mice caught outside of the animal room, we find the control series of 120 mice from the animal room yielding a spontaneous infection of $6\frac{2}{3}\%$, the experimental series, of $6\frac{2}{3}\%$ also, if we include in this series all mice whose source is indicated as doubtful in the table, which would make 90 in all. It is highly probable that all mice marked doubtful came from the animal room.

It is of interest to compare with these statistics those of the permanently infected cage. When the disease was first discovered it was thought that simple cohabitation might induce it, and hence fresh mice were introduced from time to time. To make sure of the persistence of the infection, the bodies of infected mice were placed in the cage temporarily from time to time. In Feb., 1900, a jar containing bits of infected muscular tissue mixed with bread crumbs was put into the cage. 76 days later, 6 out of the remaining 10 mice were found infected. 4 were in the earlier stages, suggesting the last feeding as the cause; 2 were in advanced stages. Whether they were also infected with younger stages was not noted. Of the 39 mice taken from this cage of which I have any records, 19 were infected, 20 free, yet all had been in the cage from 6 to 12 months and continuously exposed to mites and fecal discharges, and all had had an opportunity to infect themselves by feeding. Under these conditions about 50% became diseased, whereas in the feeding experiments the net positive result is 63.6%

The life history of *Sarcocystis muris* as interpreted by the results of the foregoing experiments consists in the invasion of the muscular system of the mouse by sporozoites taken in with the food. The sporozoites being contained in cysts cannot escape, and the infection can take place only when muscular tissue is eaten. The long period between infection and the growth of the parasites in the muscle fibres might be interpreted in two ways:

1. The parasite requires a long time to store up enough energy to undergo the rapid growth and multiple division of the nucleus terminating in the formation of spores and sporozoites. Similarly long

periods are required by the various Cysticerci of the common tapeworms of man to reach the—for them—mature stage.

2. The sporozoites after ingestion develop into sexually mature organisms in some part of the body in a manner analogous to the genus *Coccidium*. After fertilization the female organism (macrogamete) migrates into the muscle fibres and there passes through the process of sporogony.

This second hypothesis seemed the more attractive one at the outset and much time was devoted to tracing the hypothetical sexual elements in various organs of the body. Especially the intestinal tract was scrutinized carefully in animals killed at stated intervals after infection. The intestinal contents, the epithelium and the submucosa were searched in fresh preparations, in some cases in sections, for any forms which might be taken for the parasites sought for. The fate of the sporozoites soon after feeding was investigated, but no traces of them found. The spleen, kidneys and the bone-marrow, as well as the blood, received attention but nothing definite was discovered.[18]

Another view suggested itself as an outgrowth of the second. It was assumed that the sporozoites became sexually mature in the intestines, were discharged and then ingested either by the same host or other mice. This hypothesis was discredited by the impossibility of producing the disease by simple cohabitation in infected cages. Yet the occasional discovery of minute coccidia-like bodies in the intestinal contents led to a more thorough search without, however, leading to any result. The following experiment was also made. Two white mice from Expt. 18, kept together in the same jar, were transferred to fresh jars on the 2d, 3d, 4th, 6th, 8th, 9th and 11th day after feeding, to remove any hypothetical, sexually mature elements discharged with the feces. The mice were nevertheless found severely infected in due time.[19]

My observations have thus not yet bridged the gap up to the 6th week. Careful examinations of the fresh muscular tissue of mice

[18] In the spleen I encountered quite regularly the large giant cells of the bone marrow.

[19] In the digestive tract of many mice kept confined for some months and not very thrifty, two flagellates, *Lamblia intestinalis* and a trichomonas-like form were frequently encountered in the small intestine together with an amœba in the cæcum. These protozoa it was believed bear no genetic relation to *Sarcocystis muris*.

killed earlier than this have been made in large numbers, but they are not sufficient in themselves to warrant the assumption that the immigrated sporozoite is not present much earlier than this. The examination of stained sections in considerable numbers will be necessary. It may be found that the parasite reaches the muscle fibre soon after ingestion, where it remains undetected until the multiple division of the nucleus begins and the organism by its rapid growth and increased affinity for chromatin dyes becomes recognizable. The parasite reproduced in Plate I, Fig. 2, which was found 51 days after feeding, measured only .05 mm. in length and .016 mm. at its greatest width. These dimensions are four times those of the sporozoite—a very slight increase in this long period of time.

There are several aspects of this whole subject which have suggested themselves in the course of the investigation and which I shall touch upon very briefly.

The infected tissue used for feeding came, in about one-half of the experiments, from mice taken from the infected cage. In the other half, mice from one of the preceding experiments were used as indicated in the table. In the later experiments it became evident that the Sarcosporidia developed somewhat more slowly than in the earlier, and that the fully matured muscle parasites were smaller than in the earlier, and in the spontaneous infections, due not to any shrinkage in size of the sporozoites but to a smaller number. This apparent degeneration may be due to the feeding of tissue from artificial infections—a kind of artificial cultivation of the parasite under slightly abnormal conditions, such as monotonous diet and confinement of the host. It may also be due to the need of passing temporarily to some other host, or perhaps to other still unknown causes.

Can mice be infected twice or oftener, or does the first feeding establish immunity? This question was approached in one experiment. Two mice in Expt. 17, fed Nov. 25, 1900, were fed again after 55 days on Jan. 19, 1901. They were killed March 22, about 4 months after the first, 2 months after the second feeding. There was an abundant invasion due to the first feeding, but none traceable to the second feeding. Simultaneous feeding of other mice on Jan. 19 was successful.

Another question which has an important bearing on the outcome of feeding experiments is that of an initial immunity which may lead to a destruction of the sporozoites before they reach the protecting envelope of the muscular fibre. That such immunity exists is suggested but not demonstrated by the general outcome of the investigation. I have already referred to the disinclination of recently caught mice to eat muscular tissue mixed with bread, but this only partly explains the results.

May sporozoites infect when injected into the subcutis or the abdominal cavity? With the earlier feeding experiments, 5 separate tests were made to solve this problem. The results, though leaning decidedly towards the negative, were not quite satisfactory and will have to be repeated on a larger scale.

I have left the chapter on the pathology of sarcosporidiosis in the mouse, as well as the toxicity of the sporozoites, as brought out by L. Pfeiffer[20] for the parasite in sheep, and subsequently by Laveran and Mesnil[21] for the same species, for special consideration. In general the tissue reaction appears to be very slight during the developmental period of the parasite. Multiplication of muscle nuclei, scattering, small foci of leucocyte infiltration associated with slight cell proliferation of the perimysium internum and the sheath of the small vessels are usually present, but they apparently stand in no direct relation to the invasion, for the affected fibre and its immediate environment are in nearly every case normal. To interpret the slight changes present, the condition of the muscular tissue preceding visible invasion will need special attention. When the parasites are very large and numerous, as in Plate I, Fig. 1, disturbances in the movements of the animal should occur, and as a matter of fact such mice are frequently found slow in movement and ill. The sporozoites in the old cysts may shrivel and disappear and leave the chambered cysts empty as in Fig. 1. In one case I found them filled with polymorphonuclear leucocytes.

The life histories of all Sarcosporidia are not necessarily explained by the results obtained with *Sarcocystis muris*. It would be difficult, for instance, to account for the Sarcosporidia of cattle in the way those of mice can now be accounted for, since cattle are not carnivorous. Their muscle parasite is either an aberrant form from some invertebrate taken in with their food or else there is an intestinal stage as

[20] Op. cit., p. 123.

[21] *Comp. rend. Soc. de biol.*, 1899, p. 311.

well, which readily permits a discharge of spores outwards. It is obvious that other views or modifications of the views presented might be brought forward, but I desist from any further discussion not based on actual studies.[22]

The experiments described in the foregoing pages warrant the conclusion that the feeding of *Sarcocystis muris* containing ripe, mobile sporozoites to gray and white mice is followed by an invasion of the muscle fibres by the parasites, which become readily recognizable after the 45th day. At this time the parasite is, as a rule, many times larger than the sporozoite which it presumably represents. Within 2½ to 3 months the parasite has reached the final stage, in which it consists of a chambered cyst filled with sporozoites, mobile at the temperature of the body and capable of infecting other mice.

DESCRIPTION OF PLATES I-IV.

PLATE I.

The photomicrographs were made by Mr. L. S. Brown in the clinico-pathological laboratory of the Massachusetts General Hospital through the courtesy of Dr. James H. Wright, Director.

Fig. 1. Photograph. Extensive invasion of the muscular system of a gray mouse. Parasites matured, many times broader than the muscle fibres in which they originally lodged; cut obliquely. In the centre of the figure are two parasites in which only remnants of the sporozoites are present. The chambered interior is well defined. × 50.

Fig. 2. Photograph. A very early stage of *Sarcocystis muris* in the muscle fibre 51 days after feeding (Expt. No. 12). The granular appearance of the parasite is due to the presence of large numbers of nuclei. Each dark granule represents a nucleus. × 350.

PLATE II.

Fig. 3. Photograph. An early stage of *Sarcocystis muris* undergoing multiple division of the nucleus. In the two lowest nuclear areas the micronuclei are visible. Owing to the obliquity of the section only a portion of the parasite is included. From Expt. No. 9, 46 days after feeding. × 750.

Fig. 4. Photograph. An early stage from a teased preparation of fresh muscular tissue in normal salt solution. The parasite slipped out of its muscle fibre during the teasing, a frequent occurrence in this stage. The earliest division into fusiform bodies is completed. The parasitic tube is packed with fish-like bodies in some of which the refringent spherule is recognizable. × 650.

PLATE III.

Fig. 5. Photograph. Ripe sporozoites from a torn parasite. Dried lightly on a cover-glass, fixed in alcohol and ether, and stained in hæmalum. × 400.

Fig. 6. Photograph. An early stage showing the fusiform and crescentic bodies (sporoblasts or mother-sporoblasts). Hæmalum. × 625.

[22] See a paper by the writer "On a sporozoon in the intestinal villi of cattle" in Bulletin No. 3 of the *Bureau of Animal Industry*, U. S. Dept. Agriculture, p. 73. Washington, 1893.

PLATE IV.

Fig. 7. *Sarcocystis muris*, ripe stage. Natural size. From a specimen in Kaiserling's fluid.

Fig. 8. Very young stage as it appears in fresh muscle tissue in normal salt solution. The double-contoured cuticle encloses a pale, apparently structureless body substance in which refringent particles are sparingly present. ×200.

Fig. 9. A somewhat later stage under the same conditions. The refringent granules, of several sizes, are quite abundant. ×230.

Fig. 10. Stage of multiple nuclear division, corresponding to Fig. 3. To show the nuclear area, which includes the nucleus and micronucleus. From the abdominal muscles of a mouse in Expt. No. 9, 46 days after feeding. Hæmalum. For greater clearness of detail, only four are shown. Compare Fig. 3. ×1200.

Fig. 11. The stage immediately following the one characterized by the fusiform bodies of Fig. 4. The crescentic, or kidney-shaped bodies are the future sporoblasts. Each contains one or several refringent granules. Fresh preparation. ×400. (Figs. 8, 9 and 11 are from infections in Expt. 19.)

Fig. 12. The stage of sporoblasts. The latter merely outlined with the camera lucida. The outlines represent the future partitions or sporocysts. The striated margins belong to the remnants of the muscle fibres in which the parasites are lodged. From Expt. 16, 4 months after infection. Development somewhat delayed. ×280.

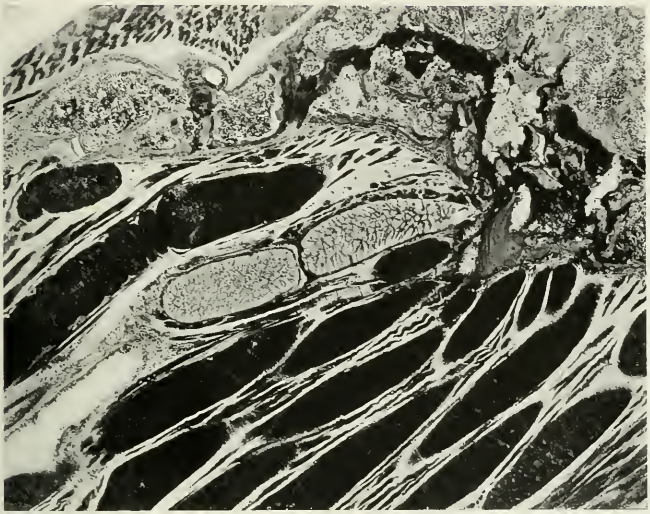


FIG. 1.



FIG. 2.



FIG. 3.

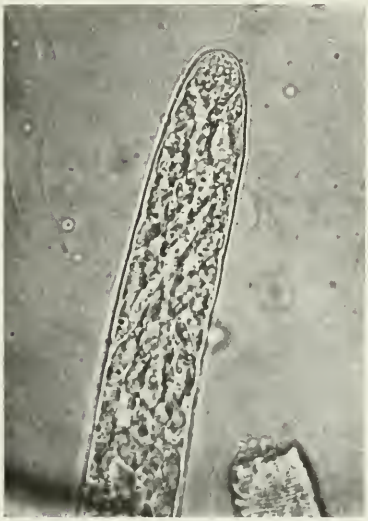


FIG. 4.



FIG. 5.

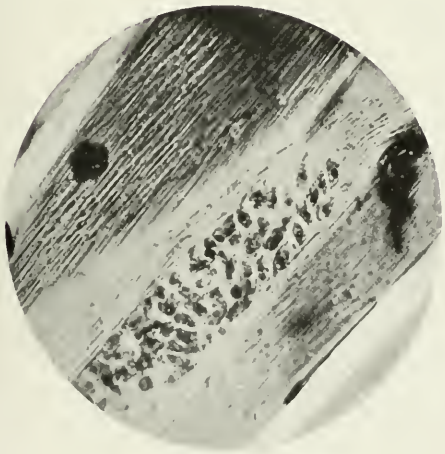


FIG. 6.



FIG. 7.



FIG. 8.



FIG. 9.



FIG. 10.

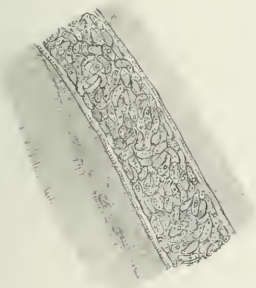


FIG. 11.

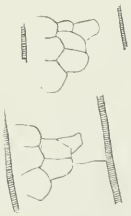


FIG. 12.

A STUDY OF CHRONIC HYPERPLASTIC TUBERCULOSIS OF THE INTESTINE, WITH REPORT OF A CASE.*

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INTRODUCTION.

During the last ten or twelve years a few writers, particularly French and German observers, have from time to time called attention to the existence of a form of tuberculosis affecting various segments of the intestinal tube and characterized by a variable but usually a considerable degree of thickening of the wall of the intestine. So definite and constant is this distinguishing characteristic that the type has been habitually referred to by French writers as *tuberculose intestinale à forme hypertrophique.* This term, however, appears to me misleading; the fundamental lesion is one of hyperplasia, hence the designation of Coquet[1]—hyperplastic tuberculosis—is certainly more correct in a pathological sense.

*An abstract of this paper was read before the New York Pathological Society, March 29, 1900.

[1] De la variété chirurgicale des tumeurs cœcales tuberculeuses. Thèse de Paris, 1894.

Ordinarily localized in the ileocæcal region, but likewise affecting other regions less frequently, the process effects a disease of long duration, the most conspicuous feature being the more or less extensive formation of fibrous and tuberculous granulation tissue in the implicated parts. This is often of such a character that the inflammatory hyperplasia or "pseudo-neoplasm" (Benoit) may easily be mistaken clinically for tumor-formation of the locality resembling carcinoma.

A condition more or less similar has been described in the larynx; its clinical presentation is also that of cancer of the part.[2] In serous membranes hyperplastic tuberculosis is also observed; the peritoneum, pleura, and especially the joints are affected in this way. Recent studies further show a more or less parallel process in a group of cases diagnosticated as Hodgkin's disease, but which fuller investigations have shown to be incited by Bacillus tuberculosis. For a knowledge of this form of tuberculous adenitis we are especially indebted to the contributions of Weishaupt,[3] Brentano and Tangl,[4] Duclion,[5] and Courmont, Tixier and Bonnet.[6] In processes of this kind the tubercle bacillus and its products induce fibrous hyperplasia rather than caseation and necrosis.

HISTORICAL.

Since the first detailed study of this peculiar type of intestinal tuberculosis by Hartmann and Pilliet[7] in 1891, it has been the source

[2] J. A. Doléris, Laryngite hypertrophique; tuberculose et sclérose partielle du poumon. *Bull. de la Soc. anat. de Paris*, 1876, li, p. 128.

Lermoyez, Végétation tuberculeuse énorme, formant polype mobile, implantée au bord de la corde vocale droite et à son insertion antérieure, etc. *Ann. d. mal. de l'oreille, du larynx*, etc., Paris, 1884, x, p. 183.

J. N. Mackenzie, Tuberkulöse Tumoren des Kehlkopfes und der Luftröhre. *Wien. med. Presse*, 1885, xxvi, p. 473.

[3] Ueber das Verhältniss von Pseudoleukämie und Tuberkulose. *Arb. a. d. path.-anat. Inst. zu Tübingen*, 1891-2, i, p. 194.

[4] Beiträge zur Aetiologie der Pseudoleukämie. *Deutsch. med. Woch.*, 1896, xvii, p. 588.

[5] Sur l'étude clinique et expérimentale de la lympho-sarcomatose et de la tuberculose hypertrophiante méconnue des ganglions lymphatiques. Thèse de Bordeaux, 1896.

[6] De la lymphadénie tuberculeuse ganglionnaire et viscérale. *Journ. de physiol. et de pathol. gén.*, 1899, i, p. 826.

[7] Notes sur une variété de typhlite tuberculeuse simulant les cancers de la région. *Bull. de la Soc. anat. de Paris*, 1891, p. 471.

of considerable interest and some discussion. To the best of my knowledge the earliest serious investigation of this anatomical type begins with these writers, the one investigating the pathological side, the other the clinical features of the disease. True, a few isolated examples of its recognition may be found in the literature prior to that date.

Thus von Hacker [8] in 1888, Suchier [9] in 1889, and Gussenbauer [10] the following year had each reported cases of this nature. As early as in 1886 Czerny [11] is said to have operated on two cases for ileocæcal disease which proved to be tuberculous; the publication of the reports, however, did not take place until 1890. Billroth,[12] in 1891, at a meeting of one of the Vienna societies, showed an amputated cæcum which had been taken out supposedly for carcinoma; the tumor was proved to be tuberculous.

But notwithstanding these earlier reports, insistence upon certain distinguishing peculiarities is the credit of the French investigators. The publication of Hartmann and Pilliet in 1891 inspired considerable interest in the subject; so that in the following two years papers on the same theme appeared by Obalinski,[13] Koenig,[14] Le Bayon,[15] Sachs,[16] Pollak,[17] Zahlmann,[18] Vohty,[19] Reclus,[20] and several others. By 1893,

[8] Ueber die Bedeutung der Anastomosenbildung am Darm für die operative Behandlung der Verengerungen desselben. *Wien. klin. Woch.*, 1888, i, pp. 359; 389.

[9] Beitrag zur operativen Behandlung der Coecumtumoren. *Berl. klin. Woch.*, 1889, p. 617.

[10] Quoted by Fink: Ueber zwei Fälle von Resection des Coecums wegen Tuberculose. *Prag. med. Woch.*, 1890, xv, p. 101.

[11] Ueber die chirurgische Behandlung intraperitonealer Tuberkulose. *Beitr. z. klin. Chir.*, 1890, xi, p. 73.

[12] *Wien. med. Presse*, 1891, p. 193.

[13] Ueber Laparotomie bei innerem Darmverschluss auf Grund eigener 110 Fälle. *Arch. f. klin. Chir.*, 1894, xlviii, p. 1.

[14] Die strictурirende Tuberculose des Darms und ihre Behandlung. *Deutsche Zeitsch. f. Chir.*, 1892, xxxiv, p. 65.

[15] De la typhlite tuberculeuse chronique. Thèse de Paris, 1892.

[16] Ein Beitrag zur Extirpation des Blinddarms wegen Tuberculose. *Arch. f. klin. Chir.*, 1892, xliii, p. 429.

[17] Ein Fall von Darmtuberculose mit schwarzem Harn. *Berl. klin. Woch.*, 1892, p. 688.

[18] Tuberkulos Tarmstriektur; resectio iutestini cœci. *Hospitalstidende*, 1892, 3. R., x, p. 901.

[19] Tuberkulos Tarmstriktur; resectio iutestini ilei. *Hospitalstidende*, 1892, No. 44.

[20] Typhlite et appendicite tuberculeuses. *Bull. méd.*, 1893, vii, p. 587.

Benoit[21] had been able to collect twenty-one such examples. Since Benoit's monograph a considerable number of new and interesting facts have been contributed by Luzzato,[22] Delbet and Mouchet,[23] Cornil and Marie,[24] Tissier,[25] Coquet,[26] Pasca,[27] Koerte,[28] Courteville,[29] Durante[30] Desplats,[31] Courtillier,[32] Caminiti Vinci,[33] Nové-Josserand,[34] Chavannaz and Carrière,[35] and a few other writers. In 1898 Itié[36] had gathered reports of forty-one new cases surgically treated, and Conrath,[37] in his paper published the same year, mentions a total of eighty-five. Itié's thesis is an admirable critique and presentation of the ensemble of the whole question; Conrath's paper contains the best general discussion of the subject, particularly in its surgical aspects. For the most part, all the cases reported relate to ileocæcal affections.

It is interesting to note how closely our knowledge of this form of intestinal tuberculosis is linked with the development of surgical intervention in the ileocæcal region.

The most recent contributions to the subject have been made by

[21] Tuberculose locale chronique de la région iléocœcale. Thèse de Paris, 1893.

[22] Tuberculosi della porzione ileo-cecale dell' intestino. *Riforma medica*, 1893, ix, pt. 1, p. 795.

[23] Rectite hypertrophique proliférante et sténosante. *Arch. gén. de méd.*, 1893, ii, pp. 513; 668.

[24] Observation de tuberculose du cæcum simulant un cancer de cet organe. *Congrès pour l'étude de la tuberculose* (1893), Paris, 1894, iii, p. 504.

[25] Infiltration embryonnaire de l'nterus, des ovaires et des tuniques de l'intestin. *Bull. Soc. anat. de Paris*, 1894, viii, p. 40.

[26] Op. cit.

[27] Tuberculosi del ileo-cæcum. *Gazz. med. di Roma*, 1894, xx, pp. 217; 385.

[28] Zur chirurgischen Behandlung der Geschwülste der Ileocöcalgegend. *Deutsche Zeitsch. f. Chir.*, 1894-5, xl, p. 562.

[29] Tuberculose avancée de la valvule ileo-cœcale; fistules, typhlite et appendicite tuberculeuse. *Jour. d. sci. méd. de Lille*, 1895, ii, p. 624.

[30] Resczioni intestini per tuberculosi de cieco. *Rif. med.*, 1895, iv, p. 616.

[31] Tuberculose pulmonaire localisée. Généralisation au pharynx et à tout le tube digestif pendant le cours d'une fièvre typhoide. *Jour. de sci. méd. de Lille*, 1896, i, p. 577.

[32] Tuberculose chronique de l'angle ileo-cœcal. *Bull. Soc. anat. de Paris*, 1896, x, p. 413.

[33] Resezione dell' intestino per tuberculosi, *Rif. med.*, 1896, No. 3, p. 112.

[34] Tuberculose localisée du cæcum traitée par la simple laparotomie. *Lyon méd.*, 1896, lxxxii, p. 154.

[35] Tuberculose du cœcum, etc. *Jour. de méd. de Bordeaux*, 1897, xxvii, p. 271.

[36] De la tuberculose intestinale à forme hypertrophique. Thèse de Montpellier, 1898.

[37] Ueber die lokale chronische Coecumtuberkulose und ihre chirurgische Behandlung. *Beiträge zur klin. Chir.*, 1898, xxi, p. 1.

Bezançon and Lapointe,[38] Pantaloni,[39] Monnier,[40] Claude,[41] Pozza,[42] Strehl,[43] Guinard,[44] Caussade and Charrier,[45] Hatch,[46] Obrastzoff,[47] and Tuffier.[48].

In English literature I have been able to find but three cases, that of Sainsbury[49] in 1892, that of Page[50] in 1897, and that of Lediard[51] reported the following year. Mention of four cases has been found in American literature—one by Beck[51a] in 1894 and one each by Ochsner[52] in 1895, by Senn[53] and by Cumston[54] in 1898.[55]

REPORT OF CASE.

The specimen of hyperplastic tuberculosis of the intestine which I was able to study is of the highest interest. The length of gut im-

[38] La tuberculose intestinale à forme hypertrophique. *Presse méd.*, 1898, i, p. 265.

[39] Résection de l'intestin grêle pour tuberculose intestinale chronique. *Arch. prov. de chir.*, 1898, vii, p. 327.

[40] Monnier. Contribution à l'étude de la tuberculose intestinale à forme hypertrophique. *Arch. prov. de méd.*, 1899, i, p. 92.

[41] Tuberculose hypertrophique non sténosante du gros intestin. *Comptes rend. Soc. de biol.*, 1898, p. 1110.

[42] Resezione dell' ansa ileo-cæcale et anastomosi terminale ileo-colica per invaginamento chronico causato da tuberculosi primitiva del cieco. Supplemento di *Policlinico*, 1899, p. 909.

[43] Ein Fall von fünfzehnfacher, zum Theil septischentzündlicher Darmstenose tuberculösen Ursprunges. *Deutsche Zeitschr. f. Chir.*, 1899, l, p. 411.

[44] Rétrécissements tuberculeux de l'intestin. *Bull. et mém. de la Soc. de chir.*, Paris, 1899, xxv, p. 327.

[45] Un cas de tuberculose ileocæcale à forme hypertrophique avec considérations cliniques, anatomiques et therapeutiques. *Arch. gén. de méd.*, 1899, i, p. 410.

[46] Tubercular disease of the cæcum. *Indian Med. Gaz.*, Calcutta, 1899, p. 43.

[47] Tuberculose d. Cæcum. *Arch. f. Verdauungskr.*, 1899, iv, p. 440.

[48] Rétrécissement tuberculeux à forme hypertrophique de l'intestin grêle. *Presse méd.*, 1900, p. 92.

[49] Primary tuberculous ulceration of large intestine. *Lancet*, 1892, ii, p. 367.

[50] Tuberculous ulceration of the cæcum giving rise to symptoms of disease of the appendix. *Lancet*, 1897, ii, p. 10.

[51] Excision of the cæcum for tuberculous disease. *Lancet*, 1898, ii, p. 408.

[51a] *Annals of Surgery*, 1894, xx, p. 672.

[52] Report of cases of resection of the cæcum. *Chicago Med. Record*, 1895, ix, p. 113.

[53] The surgical treatment of intestinal tuberculosis. *Journ. Amer. Med. Assoc.*, 1898, xxx, p. 1195.

[54] Tuberculosis of the cæcum. *Ann. of Gynec. and Pediat.*, 1898, xii, p. 29, (case iii.)

[55] Since the preparation of this article a valuable contribution to the pathology of chronic hyperplastic tuberculosis of the cæcum has been made by T. R. Crowder, (*Amer. Jour. Med. Sc.*, 1900, cxix, p. 668), who reports two new cases, in one of which carcinoma of the cæcum coëxisted.

plicated makes it unique in the records of this affection; and the absence of distinct stenosis and of apparent ulceration of the mucous membrane anywhere in the intestine adds further interest to the report. The atypical histological findings are so little suggestive of tuberculosis that this side of the study is particularly rich in interest. For the clinical history of the case I am indebted to Dr. J. L. Schoolcraft, of Schenectady, N. Y.

Clinical history.—Simon S., aged 49, was in active business until shortly after the onset of his trouble, when failing health necessitated inactivity. The family history shows no evidence of tuberculosis. When a child he suffered from some of the acute infectious diseases of this period, otherwise his health had always been excellent. No history of lues, typhoid, or dysentery.

In 1895, about three years before his death, he is said to have lost weight and to have become gradually weaker and weaker without apparent cause, so much so that walking became too fatiguing. On the slightest exertion a tendency to fainting had become manifest. Coexistent with this loss of strength there was noticed a change in the color of the skin; from a rather fair skin the color passed within a year to a dark brown. The distribution of the pigmentation was universal, not patchy, most accentuated on the exposed surfaces. Two months from the onset he began to suffer from cramp-like pains all over the abdomen, without definite localization; at times he thought them most intense in the right iliac region. Sometimes the attacks would last for only five minutes; more often, especially during the last year of his sickness, for half an hour or longer. For the most part the cramps came after irregular intervals of quiescence, at first, not over once a week; then later, four to eight times a day. Sometimes the attacks occurred shortly after taking food, more often without any reference thereto. Vomiting occurred from time to time, being often associated with the attacks of abdominal pain. Pressure had no effect in increasing or diminishing the severity of the pain. With time this whole chain of symptoms increased in severity. The appetite had been generally poor, the bowels irregular—alternating diarrhœa and constipation; there was a tendency to looseness, however, during the entire sickness. No mass was at any time felt by the patient in the abdomen, but a sensation of vague discomfort and heaviness was almost constantly present. Nothing abnormal had been observed about the stools; blood had never been noticed.

About a year and a half before his death he came under medical observation, when he was poorly nourished, of slim build, and complained of great weakness and loss of flesh. About thirty pounds in weight were lost in the first year of his sickness. The temperature was then normal; the skin of the face and hands, as well as of the trunk and body generally, was of a deep brownish color; in no wise was this patchy in character, nor was the pigmentation distinctly bronze-like. The mucous membranes of the lids and mouth were pale and free from pigmentation. The chest

was well developed, and the heart and lungs appeared normal. Examination of the abdomen revealed nothing, except that on deep palpation tenderness was elicited, seemingly most marked in the right iliac fossa; the spleen was not palpable, nor was the liver enlarged. Superficial glands nowhere enlarged. Urine normal. When seen for the first time, about 18 months before death, a diagnosis of Addison's disease was ventured. Arsenic, cod-liver oil, bone-marrow, and a very liberal and nourishing diet were prescribed, and he was sent to the country. For three or four months some improvement took place; he appeared to eat better, increased some in weight, and the abdominal disturbances improved considerably. Then the condition became more aggravated, with accentuation of all symptoms. During this time there was a fairly constant evening rise of 1 to 2 degrees F. About two months before death fainting fits occurred on the slightest exertion, until weakness necessitated remaining in bed for the last few weeks of life. Finally, some days before dissolution, symptoms of right-sided lobar pneumonia appeared and he died December 31, 1898.

Autopsy.—January 1, 1900, about twelve hours after death. The body had been injected with embalming fluid containing formalin, hence no cultures were made from the case. The notes from the protocol are as follows:

Anatomical diagnosis (revised).—*Hyperplastic tuberculosis of small and large intestine; lobar pneumonia of right lower lobe; œdema of lungs; tuberculosis of adrenals and of mesenteric lymph nodes; acute splenic tumor; cloudy swelling of liver; slight chronic diffuse nephritis.*

Body 160 cm. long, sparely built, moderately emaciated. Rigor mortis absent. No œdema of lower extremities. Surface of body universally of a dark brownish color, most pronounced on face and other exposed cutaneous surfaces. Nowhere is this patchy in character, nor suggestive of bronzing, but is more distinctly like the color produced by exposure to the sun. No pigmentation of buccal mucosa. Exposed mucous surfaces pale. Pupils moderately dilated and equal. Post-mortem lividity of dependent parts. Subcutaneous fat small in amount. Muscles of thorax and abdomen poorly developed and pale.

Peritoneal cavity.—Both layers smooth and normal. Foramen of Winslow patent. Congenital absence of appendix. Omentum delicate; omental glands not enlarged. Diaphragm, right side at fifth rib; left side at fifth interspace. Right lobe of liver reaches 5 cm. below costal margin; stomach not visible.

Both *pleural* cavities free from fluid, and *pericardium* smooth, its cavity containing a small quantity of clear yellow fluid.

Heart, small, distended with cruor clots; valves normal. Just above valves aorta shows diffuse areas of fatty atheroma. Both coronaries normal; myocardium firm, of a homogeneous red-brown color.

Left lung, free from adhesions, pleura smooth. Consistence firmer than normal. On section all lobes are brownish-red and exude a considerable quantity of blood-stained serum. No thickening at the apex.

Right lung.—Pleura smooth except over lower lobe, where it is covered with fibrin. Upper lobe contains a moderate amount of serum; lower lobe is solid, yellowish-gray in color, with streaks of brown or black, and finely

granular. No apical tuberculosis. Mucous membrane of bronchi congested. Blood-vessels normal. Bronchial glands slightly enlarged and pigmented, free from calcareous deposits or caseation.

Liver, free from adhesions, not enlarged, soft. Capsule smooth, section yellowish-brown or red in color, very cloudy.

Gall-bladder, normal, common duct patent.

Spleen, free from adhesions, moderately enlarged, firm (due to hardening fluid?), capsule smooth, trabeculæ not increased, Malphigian bodies swollen, pulp increased.

Kidneys.—Fatty capsule small in amount; fibrous capsule strips off easily; surface smooth. Cortex normal in amount; markings distinct; glomeruli visible; medulla and pelvis normal. *Ureters*, normal.

Pancreas, normal.

Left adrenal gland, enlarged, soft, and on section contains a pea-sized cavity, the walls of which are of a dark brown color and caseous, containing a small quantity of dark brownish-red fluid of viscid consistence.

Right adrenal gland, also enlarged, somewhat firmer than normal; on section shows a number of diffuse yellow areas suggestive of caseous material, but firmer in consequence of calcareous deposits.

Mesenteric glands enlarged and caseous; *retroperitoneal glands*, swollen to twice their usual size, but in them no distinct caseous areas can be made out.

Aorta, bladder, prostate, œsophagus, stomach, tongue, epiglottis, vocal cords and larynx, normal. Trachea moderately congested. Thyroid normal.

Intestine.—No adhesions. A noticeable thickening of the wall becomes apparent in the upper third of the ileum, the change from the normal intestine to the thickened ileum being so gradual as to be scarcely perceptible. Where the change occurs the intestinal wall feels harder, stiffer, until finally at the middle third of the ileum the gut becomes a cylindrical tube, with non-collapsible walls of firm, hard, resistant tissue. At a point corresponding to about the junction of the upper and middle thirds of the ileum the wall measures from 4 to 7 mm. in thickness. Passing downward, the thickening rapidly becomes more evident, especially in the last half metre of the small bowel, where the wall measures 1 cm. in thickness. The cæcum is thickened also and bound down laterally to the abdominal wall by old fibrous adhesions. Here the thickness of the wall measures 2.7 cm. The ileocolic valve is thickened and rather rigid, and shows no loss of substance. The ascending colon presents an appearance similar to the other thickened intestine, but measures not over 1.2 cm. near the cæcal end, and at the hepatic extremity only 0.75 cm. The transverse colon measures about the same (0.75 cm.); finally, the descending colon imperceptibly dimin-

ishes in thickness to the sigmoid flexure, where the bowel again assumes its normal proportions. The rectum is normal.

On section the thickened portions are resistant to the knife, and grey or whitish in color. The thickening is quite uniform in all parts of the circumference of the intestine, except in the cæcum, where it is most pronounced posteriorly; here the thickness being fully 1 cm. greater than anteriorly. To the naked eye the walls seem to be largely fibrous. No tubercles or caseous areas are visible. The lumen is entirely patent at all points, being quite normal in the ileum except in the lower half metre, where it appears reduced by about one-third. This narrowing of the lumen continues until the transverse colon is reached, whence the diameter gradually becomes more and more normal as the sigmoid is approached. The cæcum is free and the circumference of its lumen is 13.4 cm.; that of the ileum one-half metre above the ileocæcal valve is 7 cm.; that of the ascending colon 9 cm.

The mucous membranes of the duodenum and jejunum are bile-stained and smooth and normal in appearance. In the ileum the mucous membrane seems pale, and everywhere appears free from ulceration. The same is true of the remaining length of the canal. Now and again swollen solitary follicles may be seen in the small and large intestine; Peyer's patches appear unaffected. Beginning in the middle of the ileum the mucosa becomes thicker and thicker until the ileocæcal region is reached; in the cæcum and ascending colon it appears about the same and thereon becomes gradually normal.

The most conspicuous feature of the mucosa is the presence of numerous papillomatous masses, rather clubbed in form. They are most abundant in the lower ileum, cæcum and part of the ascending colon, where the largest attain as much as 8 mm. in length, the average size being about 4 or 5 mm. They are freely movable but rather rigid masses of tissue with narrow pedicles. They appear to be covered with normal mucous membrane. In the cæcum and ileum they are most numerous and quite closely aggregated; in the remaining portions they appear more discrete.

The peritoneal surface is everywhere smooth without evidence of old peritonitis, and shows no visible tubercles.

Microscopic Examination.—The tissues were placed in 95 per cent alcohol, Orth's fluid, and Müller's fluid. The sections were stained with hæmatoxylin and eosin, picric acid fuchsin, and by Weigert's modification of Gram's stain. For staining the tubercle bacilli several methods were employed, those of Ziehl-Neelsen and Kühne being particularly

used; the procedures of Letulle[56] and Hauser[57] were also used with good results. All of these methods were utilized, particularly with sections of the thickened intestine.

The histological examination of the various organs confirmed in the main the classification of the autopsy findings.

Sections of the *heart* showed a moderate degree of brown atrophy of the muscle; the pericardium and endocardium presented no change, and the cardiac blood-vessels were normal.

The *lungs* were moderately emphysematous, and, where not consolidated, showed considerable œdema with a fair amount of desquamation of the alveolar epithelium. Few red corpuscles and leucocytes were present. Sections from the *right lower lobe* showed complete consolidation by an exudate almost wholly of polymorphonuclear leucocytes; a small amount of fibrin, occasional desquamated cells, some granular material, and now and again a red blood-cell made up the remainder. On the mucous membrane of the bronchi was a small amount of granular material containing a few desquamated cells and leucocytes. The blood-vessels contained much blood in which a distinct and marked leucocytosis, polymorphonuclear in type, was discernible.

The *liver* showed albuminous degeneration, with here and there some fatty cells. The *spleen* contained considerable blood, apparently lying free between the pulp cells; the Malpighian bodies showed a lymphoid increase. Here and there within the cortex of the *kidney* tracts of round-cell infiltration were apparent, and the cells of the convoluted tubules were swollen and granular. The tufts showed occasional thickening of Bowman's capsule. In the medullary portion a few areas of round cell infiltration were also detected. Tubercles were absent.

Many sections of these organs—heart, spleen, lungs, liver and kidneys, but especially of the consolidated lung and the kidneys—were stained for tubercle bacilli, but none could be demonstrated. In sections of the pneumonic lung stained by Weigert's method were demonstrated a fair number of small round cocci often in chains (*Streptococcus pyogenes?*), and here and there among the cells large oval cocci arranged in pairs (*Micrococcus lanceolatus?*).

The *adrenals and the mesenteric lymph nodes* presented numerous tubercles and areas of caseation. Here tubercle bacilli were quite abundant. The retroperitoneal glands showed lymphoid hyperplasia and a few microscopic tubercles. Tubercle bacilli were present.

[56] *Bull. de la Soc. anat. de Paris*, vii p. 229.

[57] *Comptes rend. Soc. de biologie*, 1898, p. 1003.

The submucous tissue of the *stomach* showed some round-cell infiltration which in places penetrated into the muscular layers. No tubercle bacilli could be demonstrated within this tissue.

The histological lesions of the intestine are described more fully. (See figure in the text.)



Section of the ileum in its lower third, showing the histological features of the hyperplastic tuberculous intestine in the parts but moderately affected. The topographical and morphological details of two papillomatous processes are seen. (I am indebted to Dr. John H. Larkin for this drawing.)

Intestine.—Naked-eye examination or study with the hand lens of the stained sections of the intestine show two distinct portions, one taking the nuclear dye very strongly and including the mucous membrane and submucosa, the other staining much lighter and made up of the remaining layers.

When examined with the low powers the mucosa shows considerable thickening, about four or five times beyond the normal. In some places this is more marked than in others; it is most striking in the lower ileum and the cæcum. The epithelium generally shows little change; in places it is covered with granular material and broken-down cells, or some of the superficial cells are necrotic. Changes of this nature are, however, strictly limited to the surface. Many sections from the small and large intestine were studied, but nowhere could any solution of continuity of the mucous membrane be demonstrated. The glandular cells present no noteworthy change. The tunica propria, however, shows great increase in thickness owing to massive infiltration. When studied with higher powers this tissue is seen to be almost wholly made up of closely packed, small round cells, taking the nuclear stain deeply. Here and there oval, very few polyhedral, and some spindle cells are sprinkled among the small round cells. So great is this cellular infiltration that the gland structures have been pushed well apart; this has apparently caused diminution of their number. Occasionally wide interglandular spaces may be filled with connective tissue of the adult type in which a few round cells may be found.

The fibres of the muscularis mucosæ have been widely separated in places; at some points the cellular strands pass directly through this layer, having evidently completely destroyed the muscle fibres at these points. On the whole this layer is but moderately increased in thickness.

The papillomatous outgrowths noted at the autopsy present several points of interest. They are directly continuous with the submucous layer and present the same general character of infiltration observed there, but the round-cell accumulation is much less intense and the cellular aggregations more discrete. They are covered by an epithelial layer presenting for the most part the same general features observed in that of the intestine elsewhere. The number of unstriped muscle-fibre bundles distributed in loose and irregular strands is very striking. Just beneath the glandular layer the muscularis mucosæ may be seen as a narrow strand which contains few round cells. Generally speaking, where studied in these ingrowths, this muscle layer is quite compact, usually considerably less abundant than normal and comparatively free from any cellular infiltration. The more central parts of the papillary growths consist of bundles of adult connective tissue, rather loosely arranged, in which a moderate number of round cells are discretely spread. Among and between these connective tissue bands may be seen numerous strands of unstriped muscle tissue, quite variable in size

and compactness. The distribution is erratic. Frequently some round cells may be seen in these strands, to some extent separating the fibres, but this is never considerable. No tubercles, necrosis or giant cells were observed. As the submucosa is approached the muscular tissue becomes less evident, and the fibrous tissue and round cells become more abundant. The muscularis mucosæ becomes thinner, and is continuous with that above the submucosa proper. The whole process becomes less marked as the general level of the intestine is approached.

In the submucosa the accumulations of round cells are rather diffuse, or compact, often enough massed around blood-vessels. In some places this cellular increase completely masks the adult fibrous tissue. However, this is somewhat irregular; in some parts, particularly in the ileum, the increase of adult connective tissue appears to exceed the infiltration with round cells, and in parts of the cæcum and large bowel the same is true. The increase of round cells, however, is the predominating feature in almost all parts of the diseased intestine. In places the submucosa is fully five to eight times thicker than normal. Tubercles were not found. True, in some places, cells of an epithelioid character, intermingled with round cells were present, but these in no wise gave the impression of tubercles or of tuberculous tissue.

The outer muscular layers of the intestine present different appearances in different parts. On the whole they are everywhere thickened in the affected portions of intestine in the cæcum and colon, being three to five times thicker than normal. In the ileum, on the other hand, the degree of thickening is variable. As the cæcum is approached, it becomes greater, and here the muscular coat may be twice as thick as the normal, or even somewhat more. This thickening is due in part to muscular hypertrophy and in part to round-celled infiltration, by far the greater part being attributable to increase in the muscle fibres; the cellular increase plays but a very small part in this thickening of the muscle. Round-cell infiltration is not present in all parts of the intestinal muscle; for instance, in the ileum, except near the cæcum, it is almost entirely absent in this zone. When present the cells are either aggregated in small, irregular areas, when the muscle fibres are pushed apart or destroyed, or arranged along the course of blood-vessels. No increase of adult connective tissue was observed in this layer.

The subserosa is uniformly thickened, partly by diffuse infiltration with round cells and partly by increased adult connective tissue. The thickening is in places three times the normal. The blood-vessels here appear for the most part normal, although often enough round cells exist along their course. Thick bands of fibrous tissue, in which some

round cells are present, pass inward from the subserosa as far as the inner muscle layer, thus separating the bundles of the outer layer to a considerable extent.

The blood-vessels throughout the different layers are generally normal, but some thickening of the coats was sometimes noticeable.

Many sections from different levels were stained by several methods for tubercle bacilli. These bacilli were present in enormous numbers in the epithelial layer of the lower ileum, cæcum and ascending colon, often 20 to 40 in a field (Leitz, oc. 3, oil immersion 1/12). Higher up in the ileum and in the rest of the colon this layer contained only a few or none. The papillomatous outgrowths likewise contained only a few bacilli. In the submucosa tubercle bacilli were present in large numbers in the same regions in which they were abundant in the epithelial zone. In one section of the transverse colon where the infiltration was unusually great I was able to find a few in the outer muscular layer; here the muscle tissue was gone. In this situation the submucosa was quite free from any, so far as I could make out. Many short thick bacilli, which readily decolorized by Gram's method, were also present in the mucous membrane *(Bacillus coli communis?)*.

PATHOLOGICAL ANATOMY.

Although subject to considerable variations, hyperplastic tuberculosis of the intestine presents a fairly definite pathological picture, and on the whole exhibits certain features more or less characteristic of the process. The tumor-mass may attain great proportions and bear a striking macroscopic resemblance to carcinomatous disease, but the microscopic examination reveals the true nature of the disease. The most frequent seat of the affection, as already pointed out, is the ileocæcal region; the rectum, apparently, comes next in order of frequency. Instances of limitation of the process exclusively to the small intestine are very rare. For purposes of distinction and convenience the pathology of the condition is considered in the order of its anatomical distribution.

Ileocæcal Region and Colon.—For the most part located outside the true pelvis in the right iliac fossa these hyperplastic masses begin as small, more or less circumscribed, well-defined growths, rather nodular in form. In its fullest development the disease presents a tumor which may attain the diameter of 8 to 13 cm. Sachs[58] has seen a

[58] References to authors already cited will not be repeated.

specimen measuring 10 by 12 cm. in diameter. More often the size is much less. The process implicates the entire wall of the intestine in an annular fashion; but exceptions to this have occasionally been noted. For instance, Richelot[59] has seen an example in which the growth was situated on the posterior surface of the cæcum.

It is uncommon to find the mass entirely free from adhesions and movable; more often fibrous adhesions bind it to the abdominal wall posteriorly, sometimes to coils of intestine and other surrounding tissues. This may assume such proportions as to render operative interference impossible, as in Koerte's case. Adhesions to the anterior abdominal wall are not the rule. Occasionally the omentum becomes implicated. This has been observed by Routier[60] and Tédenat;[61] or again, as in Czerny's case, the ureter may be involved by the impinging new growth. Coquet has described a case in which the cæcum was adherent to the right kidney by a band of dense fibrous adhesions. In a case reported by Pantaloni the tumor was adherent to the urinary bladder.

In the cæcal type the hyperplastic process, as a rule, is limited to the cæcum and immediately adjoining structures. Rarely, however, quite independently of ileocæcal involvement, the transverse colon (Koenig, Eiselsberg, Esmarch) may alone be implicated. In the case reported by Claude the cæcum and ascending and descending colon were affected, while the transverse colon escaped. In a case observed by Caussade and Charrier the tuberculous process was limited to the sigmoid flexure. Involvement of the entire large bowel may occur (Pilliet and Thiéry);[62] or, as in our own case, the process may affect the greater part of the large and small intestine; this is, however, most unusual.

In the cæcal type, the tumor formed in great part of fibrous tissue infiltrated with a quantity of fat is often further enlarged by the presence of numerous lymphatic glands, some merely swollen, others

[59] Tuberculose du cæcum. Resection partielle; guérison. *Bull. et mém. Soc. de chir.*, 1892, p. 236.

[60] Vide Coquet, op. cit., p. 28.

[61] Vide Itié, op. cit. p. 12.

[62] Contribution à l'étude de la tuberculose locale du cæcum. *Progrès méd.*, 1894, 2. s., xx, p. 408.

actually caseous. A few instances of considerable lymphatic extension have been observed; in one as high as the pancreas;[63] and in another, reported by Auscher,[64] the chain of implicated glands ran up along the aorta, reaching finally to the subclavicular group. Descending glandular involvement has been noted in one instance; Coquet found the glands in the groin and Scarpa's triangle enlarged in one of his cases.

The appendix vermiformis generally escapes the tuberculous process, although it is commonly found embedded in adhesions. Sometimes it presents the ordinary lesions of simple tuberculous ulceration, caseous abscess, or miliary tubercles, or it may be almost completely lost in the new tissue and be represented only by a small finger-like cavity (Itié). Occlusion and dilatation of the lumen have been noted; in one case observed by Thiéry and Routier,[65] the appendix took part in the hyperplastic tuberculous process. In Hartmann's case the appendix was dilated.[66]

The most striking and conspicuous feature of the disease is the extremely thickened intestinal wall, which is often surrounded by a fibro-adipose adherent mass, which sometimes may be peeled or stripped from the intestine proper. "Very far from producing, as in other varieties of tuberculous ulceration of the intestine, a thinning of the walls at this level, this special form is characterized by a very marked thickening of the tunics, and moreover there is deposited around the cæcum a thick and resistant sclero-adipose mass which gives the impression of a neoplasm" (Hartmann and Pilliet). The wall of the intestine feels firm, and is of a whitish or grayish color, occasionally studded with yellow points owing to the presence of caseous tubercles, and somewhat irregular in thickness; here and there points of cicatricial contraction are apparent. The contour is consequently somewhat irregular, at other times almost cylindrical in form. The thickness of the wall varies from 0.5 to 3 cm. In one instance reported by Tédenat the thickness was 5 cm.

[63] Pilliet. Typhlite tuberculeuse chronique. *Bull. Soc. anat. de Paris*, 1891, v, p. 636.

[64] *Soc. anat.*, Nov. 29, 1895.

[65] Cited from Caussade and Charrier.

[66] Tuberculose cæcale. *Bull. Soc. anat. de Paris*, 1892, vi, p. 157.

Narrowing of the lumen at one or more places is almost constant in this class of cases; in most instances this amounts to actual stenosis; the cavity of the cæcum may be almost wholly obliterated. In the case reported by Caussade and Charrier and my own, no such stenosis was present at or near the ileocæcal valve, where it is almost invariable and greatest. The valve itself is increased in thickness and rigid from the growth of new fibrous tissue. In a majority of instances the valve exists only as an obturating membrane. It may be lost in a mass of fibrous tissue, in which a few superficial ulcers with polypoid outgrowths are present (Itié, Obs. I). The stricture may be annular, canalicular, or funnel-shaped, and is usually sufficiently marked to induce chronic intestinal obstruction as one of the clinical features. It may involve the whole length of the diseased gut, but some places are more contracted than others, especially the ileocolic junction. Occasionally the stricture is almost complete; it may be such that the cæcum scarcely admits the little finger. Becker[67] states that in his case water escaped from the constricted bowel only in drops; in Suchier's case it was even very difficult to detect the intestinal lumen.

The stenoses are produced in one of two ways: either by cicatricial contraction of tuberculous ulcers in the mucous membrane or by hyperplasia of the tissues of the wall: certain polypoid growths, already described in the record of my own case, also help such a formation. Multiple strictures are occasionally seen: Frank[68] found two, one in the ileum and the other in the ascending colon; and Czerny one, a short distance from the valve, in the ileum. Out of eighty-one cases analyzed by Conrath, strictures elsewhere than in the cæcum were found seven times. Whenever the strictures are multiple, the intervening portions of gut usually appear normal, with the exception perhaps of ulceration, but the lumen is here generally dilated. Bezançon and Lapointe have seen one case in which four constrictions were present: one just above the cæcum, the second at the hepatic flexure of the colon, the third at the left extremity of the transverse colon, the last above the rectal ampulla. Monnier reported

[67] Ueber Darmresectionen. *Deutsche Zeitsch. f. Chir.*, 1894, xxxix, p. 148.
[68] *Wien. klin. Woch.*, 1892, xxxiii, p. 1846.

another case with multiple stenoses. Strictures are not always present, as is shown by my own case and Claude's (tuberculose hypertrophique non sténosante of this writer).

In the cases with formation of ulcers the condition is merely one of association of simple tuberculous enteritis with the hyperplastic process. Compensatory hypertrophy of the ileum, often associated with dilatation and induration, follows as a result of the resistance to the progress of the intestinal contents. As insisted upon by almost all who have studied this class of lesions, this hyperplasia in the wall of the cæcum is not brought on by the constriction; this is well shown by Caussade and Charrier's case, in which the cæcum was dilated and there was no apparent stricture at any point.

The mucous membrane always presents features of considerable importance and interest. Early in the disease the mucosa may present little change. It is thickened, usually studded with ulcers and some tubercles. These may involve a considerable length of the colon and lower ileum, as in the cases of Pilliet and Thiéry, and Czerny. Cases have been reported in which the mucosa appeared intact macroscopically in well-developed forms of the disease. Such examples have been reported by Sachs, Hofmokl,[69] and Göschel;[70] in my own case no solution in the continuity of the mucous membrane could be made out anywhere, although a careful search was made both macroscopically and microscopically.[71]

Polypoid and papillomatous formations are striking features; they vary in appearance according to the cases. These structures are very constant and constitute one of the most interesting and characteristic of the gross lesions. They are very numerous and quite variable in size, being longest at the margin of ulcers. These structures occur, on the one hand, in the form of small pedunculated ingrowths; and on the other hand, instead of these small masses, or often beside them, there may be large sessile nodules or true polypoid tumors sometimes

[69] *Wien. klin. Woch.*, 1890, iii, p. 878.

[70] Ein wegen tuberculöser Strictur resecirtes Darmstück. *Münch. med. Woch.*, 1895, xlii, p. 251.

[71] In Crowder's case the mucous membrane of the hyperplastic portion was similarly free from ulceration; inasmuch as the bowel could not be examined in all its parts it is impossible to exclude ulceration in the portions above or below the section removed at operation and examined.

reaching the size of a hazelnut (Pilliet and Thiéry). They may have long, slender pedicles. The thickened mucosa, in some cases, is simply thrown into transverse folds; such a condition has been reported by Caussade and Charrier.

In the midst of these ingrowths opaque, white or yellowish white, sharply circumscribed areas appear here and there; these are caseous foci, 2 to 3 mm. in diameter, ordinarily located in the submucosa. These caseous foci, although visible, are usually separated from the lumen by the layer of glands; but they may also communicate with the lumen by a small crater-like opening. In many cases such foci are altogether absent.

Ulcers are rarely absent in all portions of the mucosa; if none are visible it does not necessarily follow that they are absent; they may well be hidden by the folds of the reduplicated mucous membrane. The ulcers are of various sizes and forms, leaving areas of intact mucous membrane between them as in ordinary tuberculous enteritis with ulceration. Sometimes they are reduced to mere crevices in the mucosa but more often they attain greater proportions and are usually annular in form. Occasionally one sees ulcers of considerable size and curious aspect.

Tuberculosis of the mesenteric lymph nodes with or without caseation is the rule. A like affection of the adrenals, as in the present case, is rare. Pulmonary tuberculosis is often secondary to the intestinal lesion in this class of cases.

Rectum.—For years hyperplastic tuberculosis of the rectum has been confused with syphilis of this viscus. Recent studies have thrown considerable light on the whole question. We are particularly indebted to Sourdille[72] for having directed attention in 1895 to this form of disease. Since then Tédenat,[73] Lapointe,[74] and Bezançon and Lapointe have further elaborated the same theme.

In a general way the lesions presented are similar to those observed elsewhere in the intestine and which have already been described.

[72] Rétrécissements cylindriques du rectum d'origine tuberculeuse. *Arch. gén. de méd.*, 1895, i, pp. 531; 697, ii. p. 44.

[73] Vide Aulès, Thèse de Montpellier, 1896.

[74] Lapointe, Traitement des rétrécissements non cougénitaux du rectum. Thèse de Paris, 1897.

The hyperplastic process is commonly observed as a cylindrical thickening of a segment of the rectum; caseous masses are not common. Tubercles may or may not be present. The number of cases thus far reported is still small; in 1897 Lapointe had succeeded in collecting but nine reports.

Small Intestine.—Hyperplastic tuberculosis of the small intestine is rare. Here it is not so often a question of those large tumor masses, so easily mistaken for carcinoma; the growth is ordinarily more limited and less voluminous. Nevertheless, the other features of the pathological and clinical pictures are present; even complete stenosis has been observed. Although the lesion of the small intestine may exist without cæcal disease, it is oftener found that the two are concomitant. In a few instances, however, the hyperplastic tuberculous disease has been confined to the ileum, the part near the cæcal end being affected.

In a case reported by Guinard, there were four stenoses; the bowel was thickened, and the lumen "dilated to the point of resembling the stomach." Perhaps the best example of the limitation of the affection to the small intestine is that reported by Pantaloni, where the ileum was affected for a distance of 12 cm., the calibre of the bowel being reduced by one-half.

When the stenosis is marked, the portion of intestine above this point is apt to show hypertrophy of the muscle tissue, and possibly some dilatation. Below the stricture atrophy and thinning of the walls may occur.

MICROSCOPIC LESIONS.

These are essentially an admixture of purely tuberculous and simple inflammatory lesions. The picture necessarily varies much according to the anatomical distribution of the lesions and the character of tissue changes induced by the tubercle bacillus or its products, and secondary infections.

The mucous membrane presents diverse alterations, according to the place examined. It is usually much thickened, sometimes measuring as much as 1 cm. The epithelium when preserved is usually normal. Here and there a few of the cells may have lost their stain-

ing properties. Elsewhere losses are perhaps apparent. The glands of Lieberkühn are absent when ulceration exists, rarely in other places. Many of the cells may have undergone mucoid or cystic degeneration.

The papillomatous outgrowths of the mucous membrane are covered with epithelial tissue, which has been thickened by round-cell infiltration. Tubercles may be found in them or in the submucosa at their base. These polypoid masses are generally continuous with the submucosa, which is often densely sprinkled with round cells; in some specimens the tissue is largely of the adult connective-tissue type. Unstriped muscle tissue may be present in abundance.

The tunica propria is likewise considerably thickened, sometimes as much as four, five or more times beyond the normal. This is due to the same character of cellular infiltration. Sometimes tubercles may be found here. The muscularis mucosæ may be missing here and there where ulceration has penetrated to the submucous layer. The muscle fibres are often widely spread apart or even destroyed by the infiltration of new cells. The thickening is most apparent in the submucosa where the fibrous increase is very marked. Pilliet states that it measured 1 cm. in his specimen; in my own the increase was also very marked. The cell infiltration is by no means uniform in its distribution or histological characters. There is often definite patchy infiltration with intervening areas of less invaded tissue. The cells are mainly of the round type; but spindle cells and much basement substance are likewise frequently to be observed. The wealth of blood-vessels occasionally seen has been studied particularly by Pilliet, and more recently by Chavannaz and Carrière. The latter observers have also noticed the presence of small hæmorrhages.

Tubercles and foci of tuberculous granulation tissue may be present in the mucous membrane; when present they are never very numerous. The majority of tubercles are found in the submucosa. Some may be caseous, with giant cells and round cells; more often the tubercles are mere aggregations of lymphoid cells in which one or more giant cells may be seen. Epithelioid cells are usually absent. The tubercles may be aggregated in small masses of sufficient size to

be appreciated by the naked eye. Many show little tendency to necrotic change; a distinct tendency to fibrous transformation is, on the other hand, apparent. The typical histological features of tubercle tissue are often absent; in lieu thereof there may exist a diffuse embryonal cell infiltration, at times capable of simulating sarcoma (Pilliet); Courtillier applies the name "lymphoid tuberculosis" to the same condition. In my specimen this appearance was well reproduced in many portions.

The muscular coat participates in the general thickening, but to a less degree than the submucosa. The muscle fibres may be widely separated by considerable cell infiltration, the latter located particularly around the blood-vessels. Occasionally tuberculous tissue may be seen in the muscular layers. Routier states that the thickening in his specimen was due largely to caseous infiltration of these layers. Disappearance of the muscle fibres has been observed in one case by Bezançon and Lapointe.

The subperitoneal layer is formed of fibrous bands in which blood-vessels may be seen showing more or less periarteritis and endarteritis; foci of round cells are observed in places. It is particularly this fibrous increase which accounts for the rigidity of the intestine in some instances. Now and then tuberculous foci may be seen, as well as some adipose tissue. Much round-cell infiltration may be present without any definite tubercles (Pilliet and Thiéry). The histological findings in my own case confirm this statement.

The serosa shows few changes in most instances, although in Hartmann and Pilliet's case it was four times as thick as the mucosa.

Most observers agree that the tubercle bacilli in the tissues are scanty; sometimes they may, however, be very abundant, as in Caussade and Charrier's case and in my own. In my specimen the bacilli were limited mainly to the inner layers, being most abundant in the submucosa; a few were present in the tunica propria. None could be demonstrated in the subperitoneal layers or serous coat.

ETIOLOGY AND PATHOGENESIS.

Knowledge of the etiological factors concerning hyperplastic tuberculosis is still unsatisfactory. The whole subject seems to be wrapped

in a certain amount of obscurity. Certain diseases have been regarded as having served as predisposing factors. Thus typhoid fever, the enteritides of infancy, and dysentery are supposed to be particularly concerned in this way. Whether or not they are to be held more responsible than some other diseases which lower the general vitality of the individual is debatable; at all events, future and more extended studies alone can decide this point. The evidence at hand scarcely justifies the conclusion that such a relationship is indisputable.

Sex evidently plays a less important rôle in favoring the development of the disease than Benoit[75] supposes. This observer believes that the disease is more common in women, but Itié's figures of a total of 71 collected cases show 40 men to 31 women. Conrath out of a total of 77 cases, found 36 men and 41 women. The influence of sex, then, does not seem to be very definite. The middle period of life gives the greatest number of cases.[76]

Occupation may have some influence, for instance, such as may lead to frequent traumatism of the right iliac region; too much stress, however, should not at present be laid on this point.

The fact that pulmonary tuberculosis was often absent in this affection, or when concomitant usually secondary in point of time, was noted by those earliest interested in this subject. This and the frequent limitation of the process to the abdominal viscera naturally suggested the primary nature of the intestinal tuberculosis. Further investigation has shown this to be apparently true for a certain number of the observed cases. But even when the clinical history seems clinching enough in this respect, the autopsy often shows "healed tuberculosis" of the lung. For this reason some writers do not concede the primal intestinal origin of the tuberculosis, but regard the bowel lesion as always secondary to some old focus in the lung or

[75] Tuberculose ileo-cæcale chronique; son traitement chirurgical. *Gaz. des hôp.*, 1898, pp. 357, 385.

[76] Crowder, (loc. cit.) gives the following table:

Age.	Male.	Female.	Total.
1 to 10	0	0	0
11 to 20	4	7	11
21 to 30	10	14	24
31 to 40	19	10	29
41 to 50	3	6	9
51 to 60	3	5	8

elsewhere. Such authors think that the occurrence of the form with subperitoneal hyperplasia further reinforces their interpretation: they regard this type as surely indicative of a hæmatogenous origin. As a matter of fact such metastasis is notoriously rare in blood infection. Conrath assumes that a revivescence of latent tuberculosis in mesenteric glands contributes to the peritoneal infection; and thence by extension the subserosa becomes invaded.

So far as I can see, the case which I have herein reported is definitely one of primary intestinal origin in the adult. Not only were evidences of pulmonary tuberculosis entirely wanting, but the character of the visceral tuberculosis in the abdomen tends further to confirm this view.[77]

Primary intestinal tuberculosis is very rare, and particularly so in the adult; the investigations of almost all pathologists clearly show this. A discussion of it, however, is foreign to the purpose of this paper. For the same reason any etiological consideration of the facts relating to intestinal tuberculosis in general has been omitted.

Simple ulcerative enteritis or colitis has been regarded as an important feature in the early genesis of the disease. Itié has particularly insisted upon this point. In his opinion the tubercle bacilli more readily implant themselves upon the mucous membrane under these conditions, and little by little transform an inflammatory process of a simple nature into one of a tuberculous character. The belief of Hartmann, Pilliet, and Benoit that secondary infections induce some of the changes described and contribute in some measure to the production of the lesions met with in this class of cases, it seems to me, will in time receive confirmation in the results of investigations in this direction. The extent to which these factors may be regarded in the pathogenesis of the disease is uncertain; they probably represent only two of the many classes of conditions which one might conceive to be of similar significance. That previous ulceration is not always necessary is shown by the case of Kirkoroff[78] and my own; in neither

[77] The first case reported by Crowder, (loc. cit., p. 671), may belong to this class; however, as no post-mortem control exists for this case objection may very properly be raised to its acceptance as such. Clinical signs, alone, invite too many errors to be accepted in this consideration.

[78] Kirkoroff. Tuberculose de l'intestin. *Arch. russes de path.*, Oct. 1898.

of these two cases was any solution of continuity demonstrable. In the case reported by me this was true for both the naked eye examination and the microscopic study. It is quite true that although no such solution could be established, one or more minute losses of tissue might exist and escape detection. But the passage of bacteria through the apparently uninjured mucosa is not wholly inconceivable; experimental investigations by Dobroklonski[79] and the writer have shown that tubercle bacilli might easily enough traverse the healthy mucosa of the intestine of rabbits (leaving it, as far as the most careful studies show, intact) and incite tuberculosis of the mesenteric glands.

The frequency with which the lesion is localized in the cæcum and rectum is probably explicable by the fact that stasis is favored in these regions, giving opportunity for implantation of the tubercle bacilli on the mucosa.

The chronicity of the disease and low grade of inflammation observed have been explained in various ways. For my own part I interpret the phenomena by assuming an infection with attenuated tubercle bacilli. Whether this is brought about, as Hartmann believes, by the intervention of secondary infections, is difficult to say. I fancy, however, that these invasions, when they occur, may have more to do with the production of simple inflammatory lesions than with an attenuation of the tubercle bacilli. The small number of bacilli present in the tissues has also been held to explain the chronic course of the process.

It is possible that bacilli of low virulence and elaborating but small quantities of toxic material irritate the tissue only sufficiently to stimulate fibrous hyperplasia, the irritant being insufficient to produce necrotic lesions. I certainly think that there is evidence for this in the case of the tubercle bacillus and *Bacillus pyocyaneus*.

Although the process is a remarkably slow one, progression finally leads to tumor-formation with resulting intestinal obstruction in a large proportion of the cases; from this the patients sink gradually to the fatal issue, unless surgical intervention is invoked. In others in which no stenosis is present some terminal infection usually carries the patient off.

[79] De la pénétration des bacilles tuberculeux dans l'organisme à travers la muqueuse intestinale. *Arch. de méd. expér. et d'anat. path.*, 1890, ii, p. 253.

Abscess formation is not common; fistulæ have been present in a few cases. Extension of the tuberculosis to the peritoneum is similarly rare. Whenever the disease progresses, it is by way of the lymph channels rather than by the blood current.

SYMPTOMATOLOGY.

Wide variations in the clinical picture are met with in different cases, determined largely by the location of the tumor-mass and the point of stenosis. The symptomatology of hyperplastic tuberculosis of the small intestine and ileocæcal region will be considered separately from that of the rectum, the clinical features of the process in these situations being sufficiently distinct to permit of separate treatment.

Hyperplastic Tuberculosis of the Small Intestine and Ileocæcal Region.—The onset is indefinite; sometimes it seems to follow some acute disease in which intestinal lesions were present, such as gastro-enteritis, dysentery, or typhoid fever. The earliest symptoms are generally referable to the gastro-intestinal canal: dyspepsia, vomiting, and some pain in the abdomen. Abdominal pain is one of the most constant symptoms at the outset, sometimes coming on after the taking of food, but usually independent of such. Colicky pains, with or without vomiting, may suddenly attack an individual in apparently good health. The abdomen may be tympanitic, with tenderness most noticeable on the right side; shooting pains through the thighs (Bouilly) may also be present. Later, or perhaps concomitant with the gastric derangement, attacks of diarrhœa and constipation often alternate, with varying prominence of the one over the other; early in the disease the former is the rule until stenosis develops. Persistent diarrhœa, in some cases without constipation at any time, has been observed (Pilliet). Constipation may be the only symptom from the start until the disease is well advanced. When marked, mechanical obstruction has probably already occurred. Hand in hand with the development of these symptoms there will be noticed loss of strength and weight, and often a slight evening rise in the temperature. These may be the only symptoms in the early part of the disease or, as sometimes occurs, throughout the illness.

The intestinal discharges present no characteristic features; at one time they may be quite normal, at another glairy and mixed with pus. Melæna has been observed in some cases. Icterus has also been noted in one case (Coquet).

With time the abdominal crises become accentuated and the weakness very great. Some patients suffer from recurring colic, accompanied by vomiting and constipation. The area of greatest tenderness is often the right iliac fossa. Meteorism is common during these attacks.

So far as I know, my case is the only one in which pigmentation of the skin has been observed; whether or not this is due to the adrenal affection, it is difficult to say. A similar grade of adrenal tuberculosis, in my experience, is often seen without any such pigmentation.

The clinical picture in the early phases of the trouble is often so indefinite that diagnosis is impossible. As the disease progresses during months or years, other symptoms of a more definite nature make their appearance. By this time signs referable to intestinal obstruction will usually draw attention to the abdominal lesion.

With the symptoms of obstruction a mass is generally noted at the point of involvement, which is usually in the right iliac fossa. From this time on the clinical picture is more and more definitely that of chronic intestinal obstruction, such as one finds in carcinoma of the same parts. This is too well known to necessitate reiteration here. If not already present, pulmonary tuberculosis may develop as a complication.

Physical Signs.—Inspection of the abdomen may show more or less distension—a condition associated at first with the abdominal crises but later becoming permanent. When there is much pain the abdomen may be contracted. The skin is usually normal. Exceptionally the size of the mass is sufficient to be seen on inspection; thus Tédenat reports one instance where it was as large as a fœtal head. A few cases show fistulous tracts, located most often in the right iliac region; openings of this kind may be found also in the vicinity of the anus. Benoit speaks of the occasional occurrence of an infiltration of the abdominal wall in this region with ulcerating fungoid growths, simulating sarcoma.

Tenderness is generally elicited by palpation wherever the growth is situated. When localized in the cæcum the limits of the tumor appear definite enough below and externally; below, the mass appears to terminate just above Poupart's ligament: laterally it lies just within the iliac crest. It rarely extends to the median line. Above, the termination may be abrupt; or the growth may continue as a cylindrical mass running up for some distance. Sometimes the decrease in size is very gradual. Esmarch, Koenig and Eiselsberg have each reported a case in which the upper limit was felt in the region of the umbilicus; here, however, the transverse colon was the seat of disease.

When the abdominal muscles are lax the growth may be felt as a firm, nodular mass, in which one can often with difficulty get lateral movement. The anterior abdominal wall, on the other hand, may be freely moved over the fixed growth.

Percussion signs are very variable and unreliable. Dullness is usually present directly over the tumor; but sometimes the note is tympanitic. In women vaginal examination may or may not be of value; rectal examination is often more satisfactory.

Rectum.—In its advanced form hyperplastic tuberculosis of the rectum presents symptoms analogous to those of other rectal stenosing diseases. Hæmorrhages from the rectum have been noted in a large proportion of the cases. They may appear from the very start, when the very earliest symptoms of inflammation begin. The bleeding may occur at defecation, or in the intervals. The blood is pure or mixed with mucus. As the disease progresses the rectal discharges change in character; instead of blood, a thick, glairy material is passed. Pain and a burning sensation in the rectum are common. Diarrhœa is sometimes present. At a later stage pain and tenderness are developed between the anus and coccyx; the sphincter ani becomes contracted, and the rectal mass is covered with many soft, pedunculated or sessile vegetations which bleed very readily. In the early stage of the affection, Itié lays a good deal of stress upon the presence around the anus of small bleeding tumors of uncertain nature.

After a period extending over months or years signs of stenosis develop. Greater and greater mechanical obstruction to the passage

of fæces is offered, and the clinical picture resembles that of syphilitic or malignant disease of this part. Perianal abscess and ulceration are sometimes formed. Examination per rectum is always painful. One or another of the divisions of the rectum may be the seat of the lesion; most often the last part of the rectum is the portion involved. The diminution of the lumen may be so great as not to permit introduction of even a sound. The entire circumference of the rectum is often thickened.

TREATMENT.

In the light of recent knowledge concerning cæcal disease, it seems reasonable to conclude that most of the resected cases of cancer of this region reported without careful microscopic examination as cured were instances of hyperplastic tuberculosis. The treatment of the condition is obviously surgical; extirpation of the diseased tissue promises good results in a fair proportion of cases. Nové-Josserand reports a recovery following simple laparotomy. Those interested in the surgical phases of this subject, including operative technique, may be referred especially to Itié's thesis and the articles by Benoit and by Conrath.

A CASE OF MULTIPLE MYELOMA.

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Plates V and VI.

During recent years there have appeared several descriptions of a peculiar condition in which simultaneously several bones become the seat of a new growth which often erodes through the cortex and pushes aside neighboring tissues, but which never metastasizes into other organs. The results of the observations of such so-called multiple myelomata and related diseases have been well summarized and discussed by Hammer,[1] Winkler,[2] Wieland,[3] Paltauf,[4] and others, and it is not necessary to repeat this discussion. In brief, the conclusions are as follows: The tumors are distinguishable from other malignant tumors by their mode of growth and the absence of metastasis—from myelogenic sarcomata in particular by their multiplicity and the uniformity of their component cells, giant cells and spindle cells being practically absent. The resemblance of the myeloma cells to those of the bone-marrow and especially the great macroscopic resemblance of the tumors to the adjacent bone-marrow, from which they can scarcely be delimited, have given rise to the opinion that they spring from some of the bone-marrow cells, although Wieland attempts to disprove this. It has been suggested that the process is of an infectious nature. Comparisons with pseudoleukæmia and leukæmia seem to show that they may be readily distinguished.

Recently Wright[5] has described in the cells of such a tumor a

[1] Virchow's *Archiv*, 1894, cxxxvii, p. 280.

[2] Virchow's *Archiv*, 1900, clxi, p. 252.

[3] Primäre multiple Sarcome der Knochen. Inaug.-Diss., Basel, 1893.

[4] *Ergebnisse der allgemeinen Pathologie u. pathologischen Anatomie.* Herausgegeben von Lubarsch u. Ostertag, 1896, iii, 1, p. 676.

[5] Contributions to the Science of Medicine, dedicated by his pupils to William Henry Welch, p. 359. Baltimore, 1900.

resemblance to plasma cells and speaks of the new growth as a plasmoma.

The cases have been described under the most various titles, but there seems to be a well-defined condition easily distinguishable from the endotheliomata and sarcomata of bone for which the name myeloma is most fitting, a condition which Virchow prophesied,[6] although at that time no case had been published.

The description of the following case may be permitted as an addition to the still rather limited series reported. The clinical features, including the occurrence of albumosuria, have already been described by Dr. Hamburger in the *Bulletin of the Johns Hopkins Hospital*, 1901, xii, p. 38. They consisted in the spontaneous occurrence of fractures of several of the bones followed by the appearance of definite soft tumor masses over various bones. There was no marked anæmia—red corpuscles, 3,548,000; leucocytes, 4500; hæmoglobin, 52 per cent, with normal relations among the different varieties of white corpuscles. Albumosuria was definitely determined.

The autopsy record, copied in brief from the protocol, is as follows:

Anatomical diagnosis.—Multiple myeloma: tumor masses in femur, ilium, clavicle, sternum and scapula, with pathological fractures. Tumor mass protruding from skull. Chronic nephritis. Arteriosclerosis. Myomata uteri. Healed tuberculosis of lungs.

Body is that of an emaciated old woman. The right leg is shorter than the left by about 3 cm., and in the trochanteric region there is a tumor mass, at which point there is excessive mobility of the femur. Tumor nodules are to be felt over the left scapula, the left clavicle, and over the vertex of the skull, where the scalp is tensely stretched over the large, soft, almost fluctuant tumor.

The organs throughout showed evidences of senile atrophy, this being especially marked in the heart and liver. The lungs present old scarred tuberculous lesions at the apices. In the uterus there were several myomatous nodules. Nowhere in the organs was there any evidence of the formation of tumors such as those to be described in the bones.

On removal of the sternum it was found to contain at the point of insertion of the second and third costal cartilages a tumor mass which,

[6] Die krankhaften Geschwülste, Bd. ii, p. 7, Berlin, 1864.

being very soft, allowed free movement of the two parts of the sternum upon one another. The left clavicle was much enlarged at its sternal end, the bone being apparently distended by the tumor mass within, for the cortical portion was very thin and could be compressed by the fingers. On sawing through the bone lengthwise the cancellous bone was found to be much rarefied and the cortical portion very much thinned—the marrow was almost entirely replaced by the tumor mass, which extended quite to the acromial end.

The right clavicle showed evidences of a healed fracture, the portions having united in a somewhat abnormal position, so that a slight angular deformity existed. The marrow of this bone also showed tumor masses, which did not, however, cause any extensive erosion of the bone.

From the spinous process of the left scapula there arose a soft tumor mass which on section was found to have eroded and replaced a considerable portion of the bony process. None of the cortex or cancellous bone tissue was to be discovered in this one. The ribs were not involved. Unfortunately the vertebral column was not sawn through, but there were no evident tumor masses visible from without. The right ilium was completely eroded through in its median portion by a large soft mass which had destroyed the whole thickness of the bone and which projected both ways—into the pelvis under the iliacus muscle, and outward under the muscles covering the outer surface of the ilium. The hip-joint on this side showed no abnormality, but in the intertrochanteric region a large tumor mass sprang from the marrow of the femur. At the upper end of the shaft of the femur there was a fracture, the shaft being displaced upward. On sawing through the bone at this point the intertrochanteric region was found to be extensively involved in the new growth, which extended into the adjacent tissues. The cancellous bone was almost entirely destroyed and the cortex much atrophied and roughened internally. For a distance of about 5 cm. the cavity of the shaft of the femur was invaded, the yellow marrow being pushed ahead and fairly sharply limited from the dark purple new growth. The bone marrow was atrophic and œdematous, greyish pink and moist in appearance, and sunken below the level of the cut surface of the invading tumor. The left femur showed no evidence of tumor formation.

Removal of the large mass at the vertex of the skull revealed a large aperture in the skull, the edges of which were very ragged, as if gnawed away, with here and there loose spicules of bone lying in the soft tumor mass which evidently sprang from the marrow cavity. This tumor mass spread itself between the cranium and the dura for a short dis-

tance, and, completely filling the aperture in the skull, projected outward to form the large soft mass felt under the scalp.

No other tumor nodules were to be found so far as it was possible to examine the bones.

These growths presented everywhere the same appearance. Everywhere they evidently sprang from the marrow of the bone from which they were not by any means sharply demarcated. Only where the tumor seemed to invade the yellow marrow of the shaft of the femur was the outline sharp, but even there the microscopical examination showed evidences of the presence of tumor elements far past this outline. Where the red marrow of the short bones formed the point of origin, the outline was not nearly so sharp. The well-defined tumor masses were perhaps somewhat firmer than such a mass of bone marrow would be. They varied somewhat in consistency however. In general they were soft; some of the larger were almost diffluent and flattened out when cut and laid out on a pan. Others were less soft, and in some parts the gelatinous pulpy consistency gave way to a fair degree of firmness. In color there was also considerable variation. The greater part of the masses was of a deep red color, perhaps even darker than that of the normal red bone marrow, but showing everywhere a greyish tint. Usually lines and streaks of grey were to be seen throughout this deep red, and in nearly all the masses definite nodules of firmer consistency and of greyish white color were found. At some points there was a slight yellow opacity.

Microscopically, the various authors have emphasized the regularity in form and size of the cells, and Wieland has adduced this as a distinction from the myelocytes. Nearly all writers have thought the tumor cells to be derived from some cell or other of the bone-marrow. Wright alone considers them to be plasma cells or closely related cells at least, to explain which he states that plasma cells are present in the bone-marrow. The results of attempts to determine the histogenesis of the cells in this case will appear from the following description of the microscopical appearances.

The tumor masses present in sections a remarkably homogeneous appearance (Plate V, Fig. 1). There is, as described in practically all of the other cases, a delicate stroma with rather wide meshes in which lie innumerable rather large round cells. These are not in intimate connection with one another, but lie singly and loose; some-

times where their number is very great they are somewhat compressed into a polygonal form, but in general they are fairly regularly rounded; they vary slightly and may be elongated or pear-shaped or even notched. The nucleus is large, round and vesicular, sometimes lying eccentrically. The protoplasm presents a rather ragged granular appearance. Blood-vessels exist throughout the tumor and are indeed rather numerous. The smaller ones lie in very intimate contact with the tumor cells, their walls being merely a single layer of endothelium. Connected with these and the coarser strands of the stroma are exceedingly fine filaments of connective tissue which run in between the cells. Everywhere scattered quite without order through the tumor mass and among the tumor cells are numerous red blood-corpuscles, which are quite well preserved. These evidently give the dark red color to the tumor masses, being absent or present in only very small quantity in the translucent greyish white nodules described above.

More careful examination of the characteristic cells of the tumor was made by the aid of various methods.

The cells are distinctly of one type, although variations in size and general appearance occur (Plate V, Fig. 1). The well-preserved cells vary in diameter from 13-21 μ. Perhaps their size is best shown by the camera drawing of a number of them where they can be compared with red corpuscles. Fig. 2 (Plate V) shows their relation in size to other well-known cells, B being tumor cells, A plasma cells, and C myelocytes. Their outline is in general smooth and sharp, and there are no processes or evidences of intimate union with adjacent cells. Ordinarily they lie quite free side by side in the spaces in the stroma, generally separated from one another by interspaces in which red corpuscles may be found.

The nucleus is quite large and rounded, and of a definitely vesicular type. It may be situated at any part of the cell, often near one extremity, when, as so frequently occurs, the cell has an elongated pear shape, this perhaps suggesting the appearance of a plasma cell. It is not at all uncommon to find two or three nuclei in one cell, and indeed four round and quite separate nuclei have been observed in a single well-preserved rounded cell. Each of these nuclei has the vesicular type and other characteristics to be described for the single ones (Plate VI, Figs. 3 and 5). In almost every intact nucleus a sharply outlined shining round nucleolus can be made out; its size is best seen in the drawings, all of which are made accurately to the same scale with the camera lucida. This nucleolus can be best seen in sections stained with carbolic fuchsin or safranin, where it stands out as

a glistening red refractive body, or with carbolthionin, where it stains bright blue and is very conspicuous. It is also to be seen in hæmatoxylin specimens, although somewhat masked by the adjacent tingible substances in the nucleus; stained with polychrome methylene blue it appears rather paler and greyer than the remaining nuclear structure.

Beside the nucleolus the nucleus contains points and strands of chromatin arranged sometimes in a somewhat radial way, sometimes more irregularly. In dried smears from the tumor mass fixed with heat and stained with Ehrlich's triple stain, the nuclei present a peculiar appearance. They look large and flattened out and stain in general a homogeneous pale blue. The nucleolus is not especially evident; it takes here a rather paler color than the remaining nuclear substance. The pale blue color does not, however, appear throughout the nucleus, for there are irregular spaces which show no blue but a reddish stain. One gets the impression that the nucleus is composed of ramifying bands of blue staining substance in the meshes of which the nuclear substance does not stain or stains only like the general protoplasm of the cell (Plate VI, Fig. 7). In this way a certain similarity exists between the staining properties of these cells and of the myelocytes. The nuclei of megaloblasts are also sometimes seen to show the same curious appearance. In the myelocytes this lacunar condition of the nucleus is quite visible, although it is somewhat masked by the presence of the granules. When the smear is stained in hæmatoxylin and eosin, however, it is very plainly to be seen. A smear from normal red bone-marrow shows many cells in addition to the granulated myelocyte, which, except in the fact that neutrophile granules are absent, resemble the myelocytes exactly, and the nuclei of these show very definitely this arrangement of fields of blue and pink.

The protoplasm appears in sections rather ragged and granular. The granules are not very sharply outlined; they are not so minute as the neutrophile granulations of polynuclear leucocytes or of myelocytes, nor so large and definite as the eosinophile granulations. Indeed one can scarcely speak of definite sharp granules, but rather of a somewhat granular appearance of the protoplasm. The raggedness is added to by the frequent occurrence of minute vacuole-like spaces, which sometimes become quite prominent. Winkler found in his case a fatty degeneration of the tumor cells and the tiny spaces in the protoplasm of these cells do suggest the presence of fat. Formaline specimens treated by Marchi's method, however, show no evidence of it.

In the sections stained with the triacid Biondi-Heidenhain mixture the protoplasmic granules appear, but they take no definite coloration

different from that of the adjacent protoplasm. In smears stained with the Ehrlich triple stain the whole protoplasm has a pale pink coloration. *No specific granulations are to be seen.*

In sections as well as in smears stained with the polychrome methylene blue of Unna or the alkaline methylene blue the protoplasm takes on only the palest greenish grey coloration; there is nothing of the specific staining described by Unna and others for the plasma cells. With polychrome methylene blue and eosin the protoplasm stains with eosin.

The relation of these cells to the other normal cells from which they might possibly arise is therefore about as follows: In size they greatly exceed the plasma cells, but agree fairly well with the myelocytes and non-granular cells resembling myelocytes found in the bone-marrow. With polychrome methylene blue, etc., they do not show the reaction typical of the plasma cells; on the other hand, their protoplasm, although in its raggedness it does resemble the "granoplasma" described by Unna for the plasma cells, shows none of the specific granulations characteristic of the myelocytes. The presence of a nucleolus must be admitted for all these various types of cells, so that it is of no help in determining such relations. The cells of the myeloma and the myelocytes and non-granular cells of the bone-marrow have in common, however, the peculiar lacunar structure of the nucleus, as seen in dried smears, which H. F. Müller[1] describes as follows: "With adequate magnification one sees in the myelocytes a remarkable nuclear structure; one finds often nuclei in which definite clear fields are visible. These may be in part nuclear substance, but in many such nuclei these fields seem to represent the cell substance which stretches itself into pre-existent holes or pores in the nucleus." And then again, "there is a large round or oval nucleus limited by a thin chromatin wall which shows frequently more or less numerous larger and smaller clear areas, which are often plainly seen to be definite apertures in the nucleus through which the cell substance extends into the interior of the nuclear body."

This structure seems so peculiar that its occurrence in these various cells at least indicates their close relation to one another. The descrip-

[1] *Deutsches Archiv f. klin. Med.* 1891, xlviii, p. 57.

tions and figures of plasma cells in the papers of Unna,[8] Jadassohn,[9] Marschalko,[10] Justi,[11] Krompecher,[12] and Councilman[13] give no hint of such a structure in the nuclei of these cells.

The myeloma cells are apparently separated from the myelocytes by the absence of the characteristic neutrophile granulations. An examination of a bone-marrow smear, and more especially of a smear from actively proliferating bone-marrow, will convince one of the great variations in the abundance of the granules which occur in these cells. In a recent paper on the relation of the myelocytes to leucocytosis, Rubinstein[14] describes the transitions which take place in the development of myelocytes from smaller cells whose protoplasm is quite free from granules. These young myelocytes reach quite the size of the adult myelocytes before the granules appear, which they do gradually a few at a time. The resemblance then between these non-granular myelocytes, as they may perhaps be called, and the myeloma cells is most striking, and suggests most strongly the origin of the myeloma from these characteristic cells of the bone-marrow in one or other stage of their development.

Further evidence of this close relation is given in the abundant presence of the tumor cells in the marrow adjacent to the tumor masses, where they take on exactly the arrangement of the myelocytes among the fat cells and are intermingled with the occasional eosinophile cells. Indeed if, in a large section, we pass gradually from the relatively normal marrow toward the tumor, we find a gradual and insensible transition, the myelocytes being replaced entirely in time by the tumor cells, which become more and more densely arranged, forming finally definite nodules. Among the trabeculæ of the cancellous bone this consolidation of the cells which have the position and form of myelocytes into solid strands in direct continuity with the tumor mass is very convincing evidence of the direct relation between the tumor and bone-marrow cells.

[8] *Monatshefte f. prakt. Dermatologie*, 1891, xli, p. 296.
[9] *Berliner klin. Wochenschrift*, 1893, xxx, p. 222.
[10] *Archiv f. Dermatologie u. Syphilis*, 1895, xxx, p. 3.
[11] Virchow's *Archiv*, 1897, cl, p. 197.
[12] Ziegler's *Beiträge z. path. Anat.*, 1898, xxiv, p. 163.
[13] *Journal of Experimental Medicine*, 1898, iii, p. 401.
[14] *Zeitsch. f. klin. Med.*, 1901, xliii, p. 161.

Various alterations in the appearance of the tumor cells, which may perhaps best be interpreted as degenerative changes, frequently occur. Division of the cells by karyokinesis has not been once observed in this case, although such nuclear figures were carefully sought after. As described above, however, many of the cells contain more than one nucleus—sometimes as many as four—and in such cases the cell is generally larger than the average, in some instances reaching a great size (Plate VI, Figs. 3 and 5). These multiple nuclei are generally rich in chromatin and stain very deeply. They are often quite irregular in outline and occasionally they are seen to be in connection with one another by a band which may be so thin as to appear as a mere filament of nuclear substance. This is very probably to be considered as evidence of amitotic division—a process demonstrated by Nedjelsky[15] in Marchand's laboratory to be not uncommon in various tumors (Plate VI, Figs. 4 and 6).

Often the nucleus of a tumor cell of average size and with the general appearance of being well preserved shows a dense clumping of its chromatin into several rounded deeply staining solid masses which lie then against the limiting membrane of the nucleus (Plate V, Fig. 1). Again, other cells show a tendency toward disintegration of the protoplasm which breaks up into fine granular masses. The nuclei of such cells often appear very pale. Finally, certain cells, sometimes of nearly the average size but generally much larger, show the presence of vacuoles of greater or less size in their somewhat swollen looking and disintegrated protoplasm. The nuclei are pale and crowded to one side, and in the vacuoles are rounded bodies showing transitions in size and appearance from very small highly refractive round bodies to rounded masses of the size of a red corpuscle or slightly larger. These are denser and more solid and refractive than the red corpuscles (Plate VI, Fig. 8). With the Biondi-Heidenhain stain, with which they show best, they stain a bright orange-yellow or brown; with polychrome methylene blue they take little or no stain, except for a rounded denser fleck in the centre, which, visible by its slight variation in color in the Biondi-Heidenhain

[15] Ziegler's *Beiträge zur path. Anat.*, 1900, xxvii, p. 431.

preparation, stains pale blue here. Such cells are often much disintegrated and the inclusions are sometimes found free. These bodies are apparently very similar to the cell inclusions so commonly found in carcinomata and other tumors, as to which there is at present such active discussion.

The tumor mass as described above contains in the interstices between the cells very numerous red blood-corpuscles in a very well-preserved condition. There is very little evidence of any breaking down of the red corpuscles—hardly any deposit of hæmatoidin in the tissues, which would certainly be present if the presence of the blood were due to actual hæmorrhage. Red corpuscles are found scattered in considerable numbers among the myelocytes and other cells in the normal bone-marrow, however, and it seems probable that the condition here is analogous. The walls of the blood-vessels in the tumor are nevertheless of extreme thinness and extravasations might readily occur.

So also tumor cells are quite frequently found inside these blood-vessels lying among the red corpuscles (Plate V, Fig. 1), although an examination of the circulating blood a few days before the death of the woman showed only one or two doubtful myelocyte-like cells among a great number of leucocytes, the varieties of which were those of the blood in practically normal relations.

In conclusion, then, we have in this case multiple new growths from the bone-marrow, not very sharply delimited from the marrow and showing very gradual transitions into it. The cells have the form and general characters of the bone-marrow cells, lacking the specific granules of the myelocytes but possessing the peculiar nuclear structure found in the myelocytes and their formative antecedents. They differ in essential particulars from the plasma cells, and in view of these facts and the fact that they largely replace the myelocytes in the marrow in the neighborhood of the tumor, there being no sharp boundary between the myeloma-like marrow and the myelocyte marrow, we may consider them directly related to these cells and probably derived from the large non-granular forerunners of the myelocytes.

Degenerative changes, the presence of numerous cell inclusions, and the abundance of red blood-cells scattered in the tumor mass have been noted. The etiology of the affection remains obscure.

DESCRIPTION OF PLATES V AND VI.

Plate V.

Fig. 1. Portion of a section of the tumor from ilium. In the centre a thin walled blood vessel in which a tumor cell lies among the blood corpuscles. Several of the cells show the condensation of the chromatin into solid masses. Among the tumor cells are many red corpuscles.

Fig. 2. *A* Plasma cells, *B* cells from the tumor, *C* myelocytes from normal bone marrow. The drawing is intended to show merely the relative size of the cells and of their nuclei.

Plate VI.

Figs. 3 and 5. Large tumor cells with multiple nuclei.

Figs. 4 and 6. Similar large cells in which the peculiar form of the nucleus is suggestive of amitotic division.

Fig. 7. Cell from myeloma as it appears in a heated smear stained with Ehrlich's triple stain. The lacunar appearance of the nucleus is exactly that seen in the myelocyte and its related cells.

Fig. 8. Several large somewhat degenerated cells with rounded inclusions lying in vacuoles in the protoplasm—one cell of the average size and appearance found in the tumor is introduced here for comparison.

All the figures except Fig. 7 are drawn with camera lucida to the same scale.

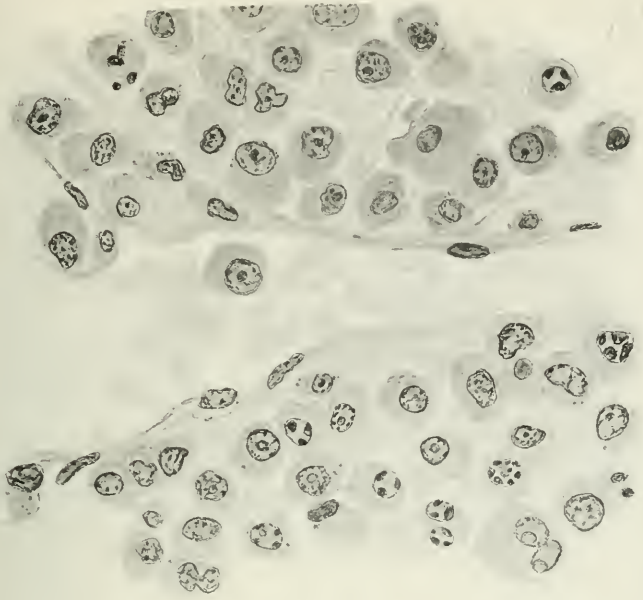


FIG. 1.

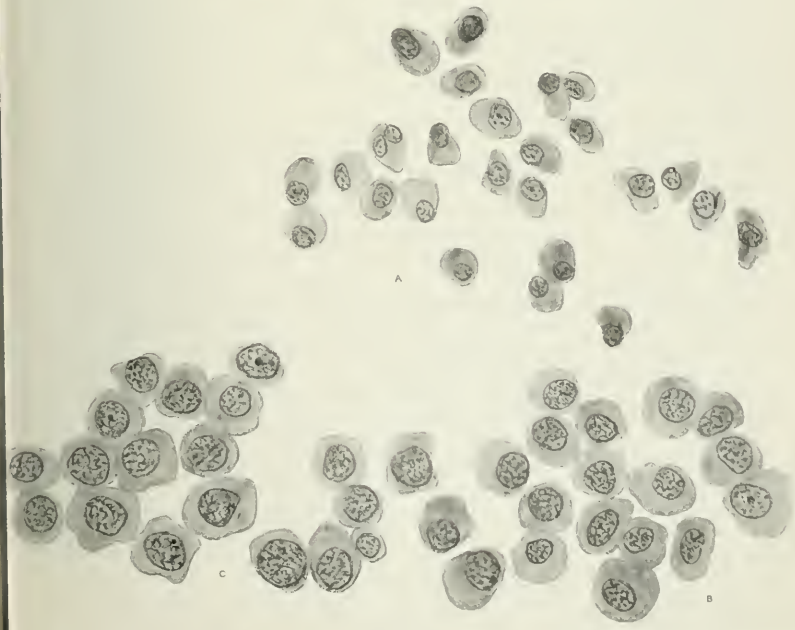


FIG. 2.

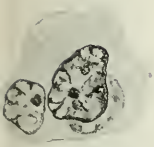


FIG. 3.



FIG. 4.



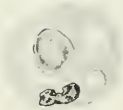
FIG. 5.



FIG. 6.



FIG. 7.









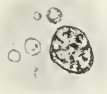
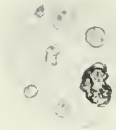


FIG. 8.



ACUTE EPIZOÖTIC LEUCOENCEPHALITIS IN HORSES.

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Plates VII and VIII.

A recent epizoötic among horses in Maryland, resulting in the death of a great many animals after a very brief illness, has led to the post-mortem examination of a number of such animals with results which seem worthy of note.

The disease, which is popularly known in this region and probably elsewhere as "cerebrospinal meningitis," presents fairly characteristic symptoms, which when the cases appear in epizoötic form lead readily enough to a diagnosis. Prodromal symptoms are not always present, although in many cases a general malaise may be noted before the acute onset. The acute symptoms are in general such as may be referred to a cerebral lesion. There may be drowsiness associated with an impairment of sight. Partial or complete paralysis of the pharynx is often observed; twitchings of the muscles of the shoulders and thighs, coldness of the extremities, and a general condition of unsteadiness and weakness with a tendency to walk to one side or a staggering, objectless gait, arise early in the disease. The pulse is usually normal; the temperature varies between 96° and 103° F., an elevated temperature usually indicating a secondary complication.

The horse may then become gradually comatose, responding slightly or not at all to stimuli and soon sinking to the stable floor not to rise again. In other cases there is a wild delirium, the animal rearing about and rushing blindly against obstacles, and this may be followed by exhaustion and the comatose condition.

The duration of the disease varies from a few hours to a week, the

average being perhaps 72 hours. Horses which recover are said to become "dummies"—animals with a permanent cerebral lesion and defective intelligence.

The following pathological report is based on the examination of four brains, brought to the laboratory by one of us (Buckley), from animals dying in the acute stages of the disease. There was also one brain from a horse which was said to have had the disease some time before and to have recovered, dying afterward from some other cause.

Of the four brains from acute cases, three were hardened in formalin and one was fresh. Of these, none showed any signs of the presence of an inflammation of the meninges; there was at most a trifling hyperæmia of the pia mater. The surface of the fresh brain showed no localized or circumscribed alterations in color, but the normal level of the convolutions was not everywhere preserved. In the frontal region on each side, anterior to the motor region of the cortex, there was a slightly depressed area which was softly fluctuant, but not marked out by any superficial hyperæmia or discoloration. On cutting through this brain a glairy fluid with small granular pulpy masses of whitish tissue flowed out from the softened area, and the rather thin roof composed of the meninges with the grey cortex collapsed over the cavity thus left. The lesion seemed almost entirely limited to the underlying white matter, which throughout an irregular area, perhaps 2 x 1 cm. in diameter in the left hemisphere, and a symmetrically placed focus 5 cm. in diameter in the right, was completely softened into a diffluent mass made up as described of shreds of softened, necrotic-looking, greyish white brain substance lying in a greyish, glairy or somewhat glutinous fluid. The portions of the brain substance forming the lining of the cavity could be fairly sharply outlined from the adjacent more normal white matter by its softness and raggedness, by its mottled greyish and yellowish opacity with translucent areas, and by the presence of numerous minute hæmorrhages sprinkled through it and adding to its mottled appearance. The remaining brain substance showed no apparent abnormality. The lining of the cerebral and olfactory ventricles was not congested nor inflamed. The blood-vessels were carefully traced and showed no thrombotic occlusion at any point.

Examined microscopically in the fresh state, the softened material showed necrotic cells and cell fragments of various forms; there were also beaded elongated fibrils thought to be axis cylinders with adhering myelin droplets. But few nuclei were found. No bacteria were found by the ordinary staining methods.

Cultures were made aërobically and anaërobically on various media—agar, glycerin agar, blood-serum agar, hydrocele-fluid agar, etc.—but all were negative. A rabbit inoculated with 1 cc. of an emulsion of the softened material into the ear vein remained well.

The appearance of the hardened brains corresponds very closely with that just described. Nowhere were any blood-vessels thrombosed or occluded in any way. Nowhere was there evidence of inflammation of the meninges. Section of the cerebral hemispheres showed irregular areas in the white matter of the occipital as well as the frontal lobes, and once in the temporal lobe, in which the brain substance had been softened and partly replaced by a translucent coagulated substance resembling agar. Shreds of greyish brain substance coursed through this clear gelatinous material. The adjacent greyish and opaque brain substance was studded with hæmorrhages through a thickness of about 3 mm. Where, as in some cases, the areas of softening were made up mainly of the greyish necrotic brain substance without much collection of fluid, the hæmorrhages were scattered throughout. In no instance did the cortical grey matter appear to be implicated, nor were the basal ganglia invaded.

Microscopically the lesions are practically identical in all the four cases except that while in all the process is quite acute, in one the destruction was less complete than in the others and the replacement of the necrotic material by coagulable fluid less extensive. A general view of a section carried through the cortex into the centre of such a focus shows the meninges practically normal, the elements of the grey cortex not notably altered, the nerve cells staining well, the blood-vessels patent and filled with blood. Passing inward the nervous elements begin rather abruptly to degenerate, disintegrate and disappear, and hæmorrhages begin to occur here and there; further toward the centre no more nerve cells are visible, axis cylinders are much degenerated, neuroglia cells stain badly, and the tissue has a much disintegrated appearance, being infiltrated with not very numerous polymorphonuclear leucocytes and fewer mononuclear round cells. Still further, and all evidences of tissue, except for small islands of necrotic substance, disappear in the highly refractive vacuolated hyaline material described (Plate VII, Fig. 1). We have then to consider in detail:

1. Changes in nervous elements.
2. Changes in neuroglia.
3. Changes in blood-vessels.
4. Changes in lymphatics.
5. Exuded fluid and cells.

The pyramidal ganglion cells which send down their axis cylinders through the degenerated area appear normal in the uninvolved portion of the cortex. The periganglionic cells may perhaps be more than usually numerous. In the lower layers as one approaches the degenerated area the ganglion cells become swollen and granular, the nucleus stains less sharply, and the cell processes, so definite in the higher layers, have been lost or disappear after a very short course, forming mere projections from the outline of the cell. Many such cells take on a rounded outline and appear now as large, irregularly rounded, granular cells with rather diffusely staining nucleus. Indeed, as in Fig. 2 (Plate VII), such cells may be seen in the same field with their disintegrating processes which are slightly separated from the cell body; others still more degenerated have lost their nuclei. The much-degenerated cells lie in a tissue of axis cylinders and neuroglia which is thickly sprinkled with globules of various sizes of high refractive index and staining faintly bluish with hæmatoxylin. In specimens stained by Weigert's method these globules take the typical myelin stain.

The axis cylinders are somewhat swollen and thick and show evidences of disintegration (Plate VII, Fig. 3). They persist, however, fairly well into the completely necrotic substance, where they end abruptly. Throughout the degenerated area their myelin sheaths are broken up into the globules described above, many of which adhering to the axis cylinders give rise to the varicose appearances or bulbous swellings along the course of the fibril. In specimens prepared by Marchi's method such varicose beaded masses often stain black.

The neuroglia has also suffered severely. Traced by the aid of Mallory's special methods from the relatively normal cortex toward the centre of an area of softening, the dense matted feltwork of the outer region is seen to give place to a delicate network of finer deeply staining fibrils, which in their turn completely disappear further toward the centre, leaving the material there without any definite neuroglia stain and consisting of necrotic debris of cells and tissue without connecting supporting substance. Associated with this gradual disintegration of the neuroglia feltwork there are changes in the neuroglia cells. These lose the sharp contours of their nucleus, which comes to stain a diffuse greyish purple without any sharply stained chromatic particles; such nuclei become more and more indistinct and finally disintegrate.

Even more striking than these destructive degenerative changes in the nervous elements and the neuroglia cells and fibrils are the changes in the blood-vessels of the affected area.

It was stated above that examination of the vessels macroscopically and with scissors failed to reveal anywhere the presence of an occluding thrombus or embolus. Sections, too, made to pass through the blood-vessels in those brains already hardened when brought to the laboratory showed them to be filled only with blood. In the area of degeneration, however, wherever small vessels are left they may sometimes be found filled or partly filled with an elongated highly refractive hyaline mass, the free ends of which may be rounded off or pass over insensibly into the adjacent compressed and coalescing red blood-corpuscles. Such hyaline formations have been found mainly in the smallest vessels and in the degenerated area. Sometimes the lumen is only partly filled and the hyaline material may show gaps in which lie red corpuscles (Plate VIII, Fig. 5), or it may form a thick bluish-staining lining for the vessel in the lumen of which lie the red corpuscles.

The walls of the vessels in these areas show, however, extensive inflammatory changes. They are infiltrated (Plate VIII, Fig. 4) with cells of the type of the polymorphonuclear leucocyte for the most part, but occasionally mononuclear or so fragmented as to be difficult of diagnosis. This process affects arteries as well as the veins, and the infiltration extends throughout all the coats. The adventitial lymphatic sheath is in most cases distended and may contain masses of polynuclear and mononuclear cells with red corpuscles. Very often, however, this sheath contains only red corpuscles, but these in such numbers as to distend it to a diameter far greater than that of the blood-vessel. It seems most probable that this hæmorrhage has occurred by diapedesis, constituting one of the evidences of inflammation, but here and there there are apparently evidences of the direct rupture of the wall of a small vessel. The distended lymph sheath may also rupture; at any rate, in nearly every case there is a zone of hæmorrhage in the tissues round about it. Such extravasated red blood-corpuscles, like those within the sheath and the blood-vessel, are in a good state of preservation, indicating the extreme acuteness of the process. There is nowhere any definite accumulation of hæmatoidin or hæmosiderin to be found in the tissues or in the lymphatics—further evidence of the rapid course of the disease.

The small vessels lying in the centre of such hæmorrhages are very commonly such as are plugged with the rather blue-staining hyaline masses already described (Plate VIII, Fig. 5). Other vessels may contain a similar hyaline material and indeed hyaline is often found both within and surrounding the vessel. Especially is this true in the case of some of the larger vessels lying within those meningeal processes

which pass deep into the sulci. There the surrounding tissue is spread apart by the presence of this coagulated material.

The nature of the hyaline substance offers perhaps some difficulty of explanation. Leyden and Goldscheider[1] express themselves as follows:

> Sometimes in œdema, softening or acute inflammation of the cord one finds in sections structureless amorphous masses. These occur in the central canal, in the grey substance, less often in the white matter, often about the vessels. This phenomenon is explained in various ways: by some thought to be coagulated albuminous or fibrinous exudate, by others interpreted as a colloid, hyaline, mucoid or gelatinous degeneration of softened nerve substance or swollen and diseased neuroglia. It is this structureless mass which Lockhart Clarke described as "granular or fluid disintegration." According to that author it consists in a softening and destruction of the nerve tissue and its change into a granular mass which, with the exuded fluid, mixes to form a homogeneous substance. These masses take the carmine stain very weakly. Their nature is not yet settled; it is even questionable whether the material under discussion is everywhere the same. The perivascular masses are most probably exudate; whether this will hold for all similar forms is, however, uncertain. The attempts to determine the nature of the substances by various stains have so far not been successful.

The problem before us is somewhat similar. The hyaline material within and about the meningeal vessels looks at times as if it had been produced by the coalescence of red corpuscles, but in general it is too abundant and homogeneous to be so explained. It is rather denser and more refractive than coagulated plasma would appear, and with water blue it stains brilliantly. In its general appearance and reaction it agrees fairly well with the larger hyaline masses in the areas of necrosis. Such hyaline material occurs also scattered about among the tissue elements, but nearly always about a vessel except in the most degenerated areas where the tissue becomes necrotic and entirely gives place to the structureless mass. There is even difficulty at times in outlining this necrotic substance from the hyaline material. Highly refractive as elsewhere it shows here, too, the tendency to contract and leave vacuoles, probably as the effect of the hardening reagent, so that the great central mass has, as a rule, an appearance almost like the cut surface of a Gruyère cheese (Plate VIII, Fig. 6). Often in such vacuoles a delicate coagulum can be made out, suggesting the presence there of a fluid of less density. The highly refractive substance is somewhat denser about the vacuoles. It is apparently very brittle in

[1] Die Erkrankungen des Rückenmarks und der Medulla oblongata, in Nothnagel's Spec. Path. u. Therap., Bd. X, Wien, 1897.

the sections and shows cracks and fissures here and there. It stains with eosin, taking a fairly bright pink color; Congo red tinges it brick red. Van Gieson's stain leaves it pinkish yellow—neither definitely red nor definitely yellow—with water blue and fuchsin it stands out sharply from the adjacent substance by its bright deep blue color; so also do the masses in and about the vessels. With Mallory's phosphotungstic acid hæmatoxylin it stains a rather pale purplish pink; with his modified stain for connective tissue as applied to the nervous system, it takes a dense deep purple color. With methylene blue, carbol fuchsin, Weigert's fibrin stain, etc., it is hardly tinged at all. Osmic acid does not stain it; in a Marchi preparation it is just visible as a smoky area.

The material stains therefore with acid dyes, in which respect (according to the hypothesis of P. Ernst) it corresponds to that form of hyaline derived from epithelial cells. Nervous elements being of epiblastic origin, might perhaps furnish the great mass of hyaline in the centre of the focus. There would be difficulty, however, in thus explaining the presence of a substance staining in exactly the same way in and about the arteries as well as the veins, and we must probably consider this one of the exceptions to the rule, as is the colloid of the thyroid which, although derived from epithelium, stains red with Van Gieson's stain.

In the smaller vessels in the neighborhood of the most intense degenerations the hyaline masses described above stain rather bluish with the hæmatoxylin and eosin stain, which seems to indicate that they are not quite identical in nature with the remaining hyaline substances described.

As stated above, the central hyaline mass in each focus is bounded by ragged edges of necrotic substance with here and there free islands of such tissue. Nowhere are there any evidences of the least pressure on this tissue, which becomes gradually rarefied toward the margin, where it quite disappears. This mass is, therefore, in all probability the result of the breaking down of the brain substance—perhaps added to also by exudation of fluid from the vessels.

The exudation of leucocytes is not very abundant in the sections. Beside the infiltration of the walls of the small vessels and the tissue surrounding them, leucocytes are found sprinkled in considerable numbers through the most degenerated tissue in the focus where it borders upon the hyaline material. These leucocytes are easily distinguished by their sharp staining from the greyish purple degenerated neuroglia nuclei which persist there.

Besides the leucocytes there are a few somewhat larger round cells with small single round nucleus and granular protoplasm. These appear to be analogous to the fat granule cells which are so common in inflammatory diseases of the nervous system of longer standing, they are however rather scarce, and although in a Marchi preparation they can be made out to contain blackened fat droplets, they are by no means a prominent feature in the section.

The process is therefore predominantly a destructive rather than an exudative one. To resume, we have an acute disease, rapidly fatal, producing large areas of complete destruction of the brain substance in which the anatomical elements are disintegrated and largely replaced by a colloid-like material. In the neighborhood the blood-vessels are acutely inflamed, there is exudation of leucocytes into the vessel walls, and throughout the adjacent tissue, with passage of the red corpuscles into the perivascular lymph sheath and into the adjacent tissues, these focal extravasations giving the inflammatory process its hæmorrhagic character.

The various forms of acute hæmorrhagic encephalitis in man as described by Wernicke, Strümpell, Friedmann and others seem, as a rule, to progress less rapidly and to be much less violently destructive than this form. Anatomically, however, the conditions are analogous.

In horses the disease is apparently fairly well recognized. Friedberger and Fröhner,[2] giving the bibliography, summarize the results of investigation into the pathology of acute encephalitis about as follows:

> Local non-purulent encephalitis occurs in irregular, round foci, mostly of the size of a pea to that of a hen's egg, sometimes even involving a whole lobe of the brain, but not sharply limited. At first the place is slightly diffusely reddened, this being soon followed by a swelling and softening from serous exudation, when, according to Schütz, the cells of the neuroglia and the ganglion cells are swollen and granular, and finally undergo fatty degeneration; the axis cylinders are varicose and the glia tissue infiltrated with small cells. The focus undergoes maceration, swelling and liquefaction, resulting finally in a softened mass consisting of disintegrated and fatty glia and ganglion cells, leucocytes and free fat-globular cells, and is spoken of as simple inflammation of the brain or inflammatory softening of the brain, distinguishable from ischæmic

[2] Lehrb. d. spec. Path. u. Therap. d. Hausthiere, Bd. ii, p. 79, 2te Aufl., Stuttgart, 1889.

encephalomalacia by the exudation of leucocytes. This may be all, but often there are complicating hæmorrhages giving rise to hæmorrhagic inflammation of the brain. With the decomposition of the hæmoglobin in such a focus the color disappears gradually and becomes yellowish. Then, as the mass of disintegrated tissue and exudate becomes more fluid, there is formed either a grey gelatinous mass or cyst, or finally a scar arises.

This description would apply to the cases described above fairly well except that the gelatinous fluid mass appears only at the end where the process is on the way to healing, whereas in our cases the brain substance throughout a large focus is quickly reduced to a gelatinous, structureless mass of necrotic and hyaline material.

The single case of our series in which recovery from the disease had occurred showed in the frontal lobe of one hemisphere a depression which on section of the brain corresponded with an elongated, grey, translucent scar which ran deep into the substance of the brain. This microscopically showed only a loose granulation tissue with numerous cells resembling the fat granule cells. Of course, whether or not it was really the end product of such a condition as described above depends on the accuracy of the diagnosis, but as the symptoms are fairly characteristic and the scarred condition of the brain about what might be expected as the final result of the anatomical process, it seems probable that this was an instance of recovery from the affection here described.

Addendum.—Since the above was sent to press there has occurred another outbreak of the disease in southern Maryland in the course of which great numbers of horses have died. We were able to make three autopsies on animals in which the symptoms during life were such as are described above. The two horses when seen were comatose while the third animal—a mule—had died after a short but violent delirium. As the horses were obviously dying they were killed, but the autopsies revealed no recognizable macroscopic lesion. Microscopically, however, the vessels in the substance of the brain show in many places an acute inflammatory affection of and around their walls, and here and there in their neighborhood there is infiltration of the tissue with mononuclear, polymorphonuclear and eosinophilic leucocytes. No widespread destruction such as that described for the previous cases was found in these cases, and it is clear that they represent an earlier stage of the affection than that described above.

Bacteriological examination in these cases led also to no satisfactory results. Cultures from the organs of the horses were sterile except for occasional obvious contaminations. A rabbit inoculated with an emulsion of the brain substance of the mule, which had been dead 48 hours, died with a general infection with a bacillus probably of the hog-cholera group and very virulent to rabbits. Further study of this organism will be made but it is not likely that it has any relation to the disease in question.

DESCRIPTION OF PLATES VII AND VIII.

PLATE VII.

Fig. 1. Photograph of a section through part of a focus of encephalitis showing the disintegration of the white matter, and the central hyaline substance.

Fig. 2. Ganglion cells which are losing their processes and becoming rounded—steps toward their complete disintegration.

Fig. 3. Nerve fibres undergoing degeneration. The myelin sheath forms droplets or varicosities along the axis cylinder. Other highly refractive droplets are scattered about in the tissue.

PLATE VIII.

Fig. 4. Small vessel with cellular infiltration of the wall, the perivascular lymph sheath being distended with blood.

Fig. 5. Similar vessel with extravasation of blood into its lymph sheath. The vessel is partly filled with a hyaline material.

Fig. 6. Central portion of a large focus showing the margin of the necrotic material and the central hyaline substance with vacuoles.



FIG. 1.

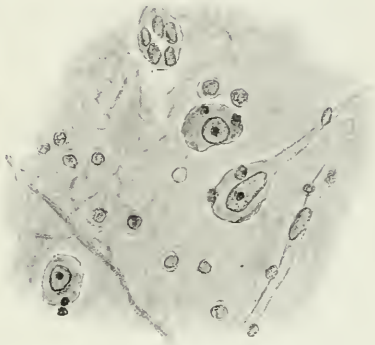


FIG. 2



FIG. 3.

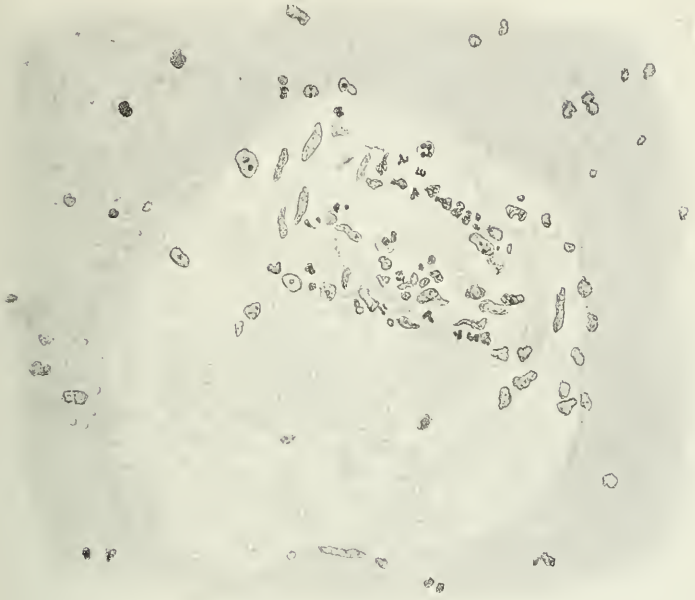


FIG. 4.

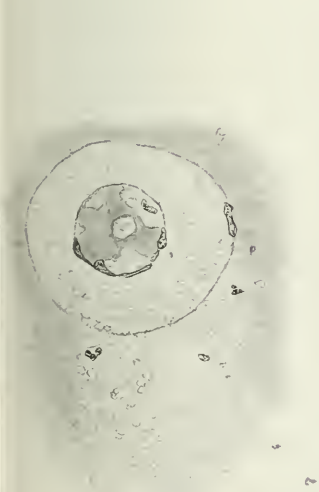


FIG. 5.

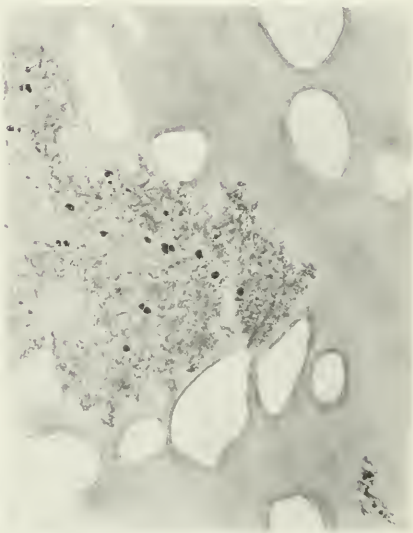


FIG. 6.

ON THE OCCURRENCE OF STRONGYLOIDES INTESTINALIS IN THE UNITED STATES.

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PLATE IX.

HISTORICAL.

In 1876 Normand (1876),* a French naval surgeon, discovered a small nematode in the dejecta of patients who had contracted severe diarrhœa in Cochin China. This parasite, present in enormous numbers, was afterwards studied more carefully by Bavay (1876), who described it as *Anguillula stercoralis*. The worm, according to Bavay, differs but little from the terrestrial anguillula, *Rhabditis terricola* Dujardin, genus *Leptodera* of Schneider. The parasites were usually met with, in the stools, as larvæ, measuring about 0.33 x 0.022 mm. in size. When the stools were kept in uncovered vessels at a sufficiently warm temperature, these larvæ underwent development, reaching full growth and sexual differentiation in about five days. The length of the full grown female was about 1 mm.; its breadth about 0.04. The body was cylindrical, slightly diminishing in size anteriorly and tapering to a sharp point posteriorly. When the worm retracted forcibly, slight transverse furrows were to be seen. The mouth as described by Bavay, possessed three distinct lips, and was continuous with the triangular œsophagus, which, after a stricture, dilated again into a second ovoid enlargement. The intestine, which followed, was fairly visible, and ended in a little protrusion on one side of the body near the base of the tail. The intestine was pushed slightly aside by the uterus. A little below the middle of the body, and on the ventral side, opened the vulva, leading to the uterus, which extended from the intestinal ventricle to a point near the anus. Here the eggs were massed in varying number. In some instances the young had actually broken the shell of the eggs, and were free in the uterus, though more often the eggs, on deposition, contained well formed, motile embryos.

* References to authors are arranged alphabetically at the end of this article.

The male was about one-fifth smaller than the female. The perintestinal cells were more clearly outlined, and were accompanied by another long gland, which seemed to consist of small rounded globules. This organ, doubtless the testicle, ended at the base of the tail in two small, horn-like spicules with tapering extremities curved inward; these spicules contained canals. They were of equal length, and were situated symmetrically on a transverse plane. The tail, which was coiled also in the same direction as the spicules, was twice as short as that of the female. The authors further described copulation, the laying of eggs and the development of larvæ. Neither Normand nor Bavay were able to cultivate the adult animal from the second generation.

In the following year Normand (1877) discovered a second nematode, present, in association with these parasites, in the small intestine of a patient dying from Cochin China diarrhœa. Bavay (1877[1, 2]), who afterwards found this parasite in a number of autopsies upon similar cases, described it as a distinct species, which he termed *Anguillula intestinalis*. It was met with only in the female form, the length of which was 2.20 mm.; the average breadth 0.03. The body, a little tapering anteriorly, terminated, rather suddenly, posteriorly in a conical tail, the extremity of which was appreciably rounded, and even a trifle dilated. With a sufficient magnification, the surface showed a very delicate transverse striation. The mouth, without horny armature, showed three small lips. It opened into an œsophagus, practically cylindrical, which occupied about one-fourth of the length of the animal, and showed neither swellings nor striations. It was followed by an intestine, with which one might readily confound it, without a marked change of color. This intestine extended nearly to the posterior extremity of the body, but it almost ceased to be visible in the middle part, which was occupied by a very large, elongated ovary. The vulva was situated in the posterior third of the animal, and, in its neighborhood, the uterus contained usually five or six rather elongated eggs. The anus, a transverse slit, was situated toward the base of the tail. The eggs and the viscera were of a yellowish green color, rather opaque and apparently finely granular. All individuals observed were oviparous females. Bavay questioned whether the absence of males was due to their prompt disappearance after coupling, or whether, as Schneider has shown to be the case in certain nematodes, the worm is unisexual when free, and hermaphroditic with a female habitus when parasitic.

These worms were abundant in the duodenum, rarer in the jejunum;

they were not found as far down as the ileum. Once, they were found in considerable numbers with embryos of *A. stercoralis* in fluid coming from the stomach. In intestines where the worm was found, it was not uncommon to find also series of eggs, often joined together, sometimes isolated. In some of these the embryo, in the process of formation, showed a definite row of dorsal cells; in others its development was more advanced.

These parasites were hardly ever found in the stools. Bavay (1877 [2]) found the worm in six cases, and in five of these only at autopsy. The development of the eggs was not observed, but Bavay (1877 [2]) makes the following statement:

"In three diarrhœic stools which we had preserved to follow the development of the *Anguillula stercoralis*, we found, at the end of several days, that they contained larvæ different from the first. These were, as a matter of fact, longer, with a cylindrical œsophagus descending down to about the middle of the body, and a tail, which, instead of terminating in a fine point, was, on the contrary, apparently truncated at its extremity. Although the culture of these larvæ could not be carried far enough to establish in an irrefutable fashion their identity with the *Anguillula intestinalis*, yet we had no doubt with regard to this point. Indeed, two of the patients who presented this form in their stools, have succumbed since then, and the autopsy has shown us the complete form. The third case still lives."

Both Normand and Bavay were inclined to regard the parasites, especially *Anguillula stercoralis*, as important etiological factors in Cochin China diarrhœa.

These observations were partly confirmed in 1877 and 1878 by Laveran (1877 [1, 2]), Libermann (1877 [1, 2]), Roux (1877) and Chastang (1878) in patients from China, and by Chauvin (1878) in a case originating in Martinique. Their observations related almost entirely to the discovery of rhabditiform embryos in the stools. Chauvin, however, states that he was able, in cultures, to follow the deposition of the eggs by the adult female, and that he noted the presence, together with the embryos of the new generation, of a longer, thinner and more motile worm, "probably that which has been mentioned by Normand."*

In 1878 Grassi (1878, 1879 [1, 2]) and Parona (1879) discovered *A. intestinalis* at a number of autopsies in Pavia, and published a careful description of the mother worm, of the eggs and of the embryos developing from them.

The mother worm was found throughout the upper gastro-intestinal tract, especially in the lower part of the duodenum and the upper

* Normand (1877) quotes Bavay (1877 [2]); vide supra.

part of the jejunum, though they were occasionally met with throughout the jejunum and in the upper part of the ileum. They have been found in the stomach. In some cases the intestinal mucosa appeared to be perfectly healthy. The eggs, deposited in the intestinal tract, hatched almost immediately after being laid. They were found, as a rule, in close proximity to the mother worm. It was extremely rare to find the eggs in the stools. The embryos in the fæces, which were almost exactly similar to those described by Normand and Bavay as *A. stercoralis*, were identical with those found in the intestine. In cultures kept at room temperature in July, the fæces being moistened with a little water, these worms were found to grow gradually, until, after four or five days, sometimes longer, they measured about 0.6, while the breadth diminished to about 0.015. The two bulbous enlargements disappeared from the œsophagus, which occupied more than a third of the entire length of the animal, and was bordered by granulations. The genital rudiment had disappeared. After ten or twelve days, many of the worms were 0.75 long, but the longest were only about 0.029 or 0.03 in width; the œsophagus occupied one-half the length of the animal.

It was pointed out that, at this stage, the embryos were similar to those forms observed by Bavay and Chauvin in fæces which had been kept for some time; forms which Bavay (1872 [5]) had believed to be larvæ of *A. intestinalis*.

They failed to observe in their cultures, at autopsy, or in the stools, a single sexually differentiated *A. stercoralis*.

Grassi (1879 [1]) placed the worm in a special genus, closely allied to *Strongylus*, which he termed *Strongyloides*, and later (1879 [2]) he suggests the specific term *Strongyloides intestinalis*. This classification has been accepted by the best authorities.

At the same time Perroncito (1880 [1-6], 1881, 1882, 1883) called attention to the presence of *Anchylostoma duodenale* and also *Anguillula intestinalis* and *A. stercoralis* in the intestinal tract of workers in the St. Gothard tunnel. He (1881, 1882) succeeding in cultivating, from rhabditiform embryos found in the fresh fæces, adult *Anguillula stercoralis*, which he termed *Pseudo-rhabditis stercoralis* (Bavay). The original rhabditiform embryos were, from his descriptions, essentially similar to those described by Normand, Bavay, Grassi and Parona. He described and pictured with accuracy the development of the adult male and female forms from the rhabditiform embryos in the stools, their copulation, the laying of eggs, the hatching of these, and the gradual transition of the young embryos of the second generation into filariform larvæ,

very closely similar to those described by Grassi and Parona as the final stage of the metamorphosis of the rhabditiform embryos, the descent of which from *Anguillula intestinalis* they had traced.

Perroncito also described *A. intestinalis*, but contrary to the observations of Grassi and Parona, he asserted that the eggs of this worm appeared in great numbers in the stools, and were, at some stages, indistinguishable from those of *Anchylostoma duodenale*. In cultures, the larvæ developing from these eggs were closely similar to those of *Anchylostoma*, the differences described being extremely slight.

The differences between the larvæ of *Anguillula intestinalis* and *A. stercoralis*, Perroncito believed to be slight but yet characteristic. He maintained, however, that the larvæ of *A. intestinalis* and *Anchylostoma* were never found in recent fæces. In his own words: "This is sufficient of itself to establish the diagnosis" (1880 [3]).

These observations were followed by a rather spirited controversy, Grassi (1882, 1883 [1, 2, 3]) maintaining, and proving beyond a doubt, that Perroncito had fallen into error in assuming that the eggs of *A. intestinalis* were passed in any number in the fæces. He showed, clearly, that Perroncito had been dealing purely with *Anchylostoma* eggs. He insisted that the rhabditiform embryos found in the dejecta were direct descendants of the strongyloid so-called *Anguillula intestinalis*, and, in view of the apparently clear evidence that these embryos often develop, in cultures in the free state, into sexually differentiated adult parasites, so-called *Anguillula stercoralis*, he advanced, in 1882, the hypothesis, based upon a number of observations, that *Anguillula intestinalis* was a dimorphobiotic parasite like *Ascaris nigrovenosa*; that the mother worm in the intestine was parthenogenetic or hermaphroditic; that the rhabditiform embryos developing from the eggs of these and escaping in the dejecta, developed into a sexually differentiated generation outside of the body; that the descendants of this sex-ripe generation were capable, after ingestion, of developing again, into the parthenogenetic or hermaphroditic parasitic mother worm.

In the same year there occurred, in the clinic of Gerhardt at Würzburg, a case which was carefully studied by Seifert (1883 [1, 2]), Grassi, and Leuckart (1883), which was destined to shed much light upon this disputed question. Seifert sent specimens of the dejecta to Leuckart in Leipzig. These contained great numbers of typical rhabditiform embryos, unquestionably similar to those described by Normand, Bavay, Perroncito on the one hand, and by Grassi and Parona on the other. In cultures, these developed into sexually differentiated *Anguillula stercoralis*. The new embryos developing from the eggs of these under-

went the course of development described by Normand, Bavay and Perroncito, changing, eventually, into characteristic filariform larvæ. Leuckart was convinced that these filariform larvæ must pass over into a suitable host to return to complete development and sexual maturity, and from analogy with what he had previously observed in *Ascaris nigrovenosa*, he agreed with Grassi in assuming that these various forms were but different phases in the cycle of a single heterogenic parasite. "The structure of the filariform larvæ is such that they cannot possibly develop again into the rhabditis form. There must be another worm which develops from them, a variety which, in the shape of its body and the structure of the œsophagus resembles these." And *Anguillula intestinalis* possesses these characters. Owing to the entire absence of males he believed, as did Grassi, that the parasitic mother worm, of female habitus, was hermaphroditic or parthenogenetic. While he expressed no positive opinion, he was inclined to suspect that, as Schneider had shown to be the case in the analogous stage of *Ascaris nigrovenosa*, the so-called *Anguillula intestinalis* was hermaphroditic.

More recently, however, Rovelli (1888), who has investigated this question, has come to the conclusion that the worm is parthenogenetic.

Leuckart was strengthened in his views concerning the life history of the parasites by the fact that Grassi and Parona (1879) had demonstrated the origin of the rhabditiform embryos found in the dejecta from *A. intestinalis*, while both Bavay and Grassi (also Chauvin (1878)) had described filariform larvæ in old cultures. He suggested for the parasite the name *Rhabdonema strongyloides*, and in conclusion says: "The *Rhabditis stercoralis* itself is to be erased from the list of essential parasites; it represents, like the *Rhabditis ascaridis nigrovenosa*, despite its sexual differentiation, an intermediate generation, developing externally, which forms a link in the chain of development of the *Anguillula intestinalis*."

At the suggestion of Grassi, who also observed this case, the patient was given on several occasions large doses of male fern, santonin and thymol. On one occasion, after an anthelmintic followed by a purge, two examples of the mother worm were recognized by Grassi (Seifert, 1883 [2]) in the fæces; both, however, were dead and had undergone considerable post-mortem change. On another occasion, after administration of apomorphia, a considerably degenerated *A. intestinalis* was found in the vomitus. No examples of sexually differentiated adult *A. stercoralis* were ever observed in the fresh stools.

In the same year Grassi (1883 [1]) emphasized the interesting fact that filariform larvæ, identical with those into which the primarily rhabditi-

form embryos of the free living generation develop, may arise by *direct* transformation from the rhabditiform embryos of the parasitic mother worm, namely, the embryos found ordinarily in the dejecta. This direct transformation had been clearly described by Grassi and Parona (1879) four years before, and in the experience of the former, represents the ordinary method of development.

In support of these observations showing that the rhabditiform embryos of the parasitic mother worm may, under some circumstances, change directly into the filariform larvæ, without the interposition of the sexually differentiated free living generation, Grassi (1883 [1]) notes the fact that exacerbations of the infection in patients who have been living in regions where fresh infection by the mouth is out of the question, are not uncommon. He also points out that it is not infrequent to find, in the cadavers of individuals who have remained in hospitals for several months, small and rather immature examples of *A. intestinalis*. Such a parasite was passed by the Würzburg patient. There is no evidence that the sex-ripe intermediate generation ever develops in the intestinal tract during life. Such forms are never found at autopsy. The presence, then, of immature forms of *A. intestinalis* at autopsy, and the increase in the number of embryos in the stools during life, must depend upon a direct transformation of the rhabditiform embryos into the mother worm without the interpolation of the sexually differentiated generation.

In 1884, Golgi and Monti (1884, 1885) made a careful study of this question in cases observed in Pavia. They confirmed *in toto* the observations of Grassi and Parona, and agreed with these observers in pointing out Perroncito's error in assuming that the sexually differentiated, free living generation is a separate parasite. They followed in cultures the direct transformation of the rhabditiform embryos of the parasitic mother worm into the filariform larvæ, as well as the indirect change through the free living, sexually differentiated generation. They agreed with Grassi in believing the former cycle to be the commoner.

In the meantime a number of other observers had described cases in which this worm was present: Breton (1879) in China; Sahli (1882) and Bozzolo and Pagliani (1880) in cases from the St. Gothard Tunnel, Ribeiro da Luz (1880) and Lutz (1885) in Brazil; Radetski (1886) in Russia; while Grassi (1878), Grassi and Calandruccio (1884), Grassi and Segrè (1887), Lutz (1885), and others described similar parasites in other animals. Since then Calandruccio (1889 [1, 2]) and Barbagallo (1897) have found the parasite in Sicily; Sonsino (1889, 1891), Riva (1891) and de Silvestri (1895) in Italy; Ilberg (1892) in a case from the

Dutch Indies; Sonsino (1896) in Egypt; while Leichtenstern (1898, 1899) and Wilms (1897) have described a number of cases occurring especially in brickworkers along the Rhine, and Poppenheim (1899), a sporadic case in East Prussia. Pérez Valdés (1897) has observed the parasite in Spain, and Strong (1901) in the Philippine Islands.

The most important work of recent years has been done in the clinic of Leichtenstern. As a result of fourteen years' study of fourteen cases, this observer comes to the following conclusions (1898):

"(1) The direct metamorphosis of the Anguillula embryos into the filariform is the rule. In some of my patients with Anguillula, this method of development was observed exclusively for weeks and months, however one might vary the conditions under which the cultures were made.

"(2) The development of the sex-ripe intermediate generation, *Rhabditis stercoralis*, takes place commonly, but by far not so constantly and regularly as the direct metamorphosis.

"(3) In those cases again, which, in my experience, are unusual, where the development of the sex-ripe intermediate generation predominated continually or for transient periods, I have never failed to observe the direct metamorphosis as well.

"It is then a matter of purely facultative, by no means exclusive or obligatory heterogenicity."

As to the reason why, in one instance, the direct method of transformation should prevail, and in another the indirect, there has been much question. Wilms (1897), in Leichtenstern's laboratory, has proved definitely that it is not due to the existence of two distinct varieties of worm. This observer administered to human beings filariform larvæ which had developed by the method of direct transformation from rhabditiform embryos of the parasitic mother worm. After seventeen days, rhabditiform embryos began to appear in the patient's stools. In culture experiments these characteristic rhabditiform embryos underwent, in part, a direct metamorphosis into new filariform larvæ, but in part, developed into the sexually differentiated, free living generation, the so-called *Rhabditis stercoralis*, from the eggs of which, in turn, there arose rhabditiform embryos changing rapidly into filariform larvæ.

Experiments with culture media, variations in the temperature, moisture, etc., to which the cultures are exposed, have failed to reveal any definite law as to the reason for the prevalence of one or another of these methods of development. Leichtenstern (1899), in his last article, has pointed out the fact that the sex-ripe intermediate genera-

tion is apparently commonest in cases which have been but recently imported from the tropics, while the direct metamorphosis is the rule among those instances originating in Italy, Belgium, Germany and Holland. It is, however, probable that all these worms were ultimately of tropical origin. So that one may be led to believe that the parasite, after entering into temperate regions, has adapted itself to the less favorable climatic outward conditions, in that in temperature zones, it tends to follow the direct method of transformation into the filariform larvæ, a method which is simpler, more rapid and less dependent upon outward influences. And yet, despite this, in any given case there may, for a certain time, occur a change, as a result of which the development of the sexually differentiated intermediate generation prevails. The cause of such variations in the type of the cycle of development is quite obscure.

The only observer of recent years who has failed to recognize the heterogenicity of the parasite is Teissier (1895 [1, 2]), who in 1895 reported a remarkable instance in which the stools contained worms which he believed to be identical with the rhabditiform embryos above described. In cultures they developed into sexually differentiated free living parasites closely similar to those described by Bavay, Perroncito, Grassi and others. In the circulating blood, however, there were also found numerous small larvæ which he believed to be the earliest stages of these embryos. Contrary to the experience of almost all other observers, Teissier found adult, sexually differentiated forms, not only in cultures, but also in the fresh dejecta, and concludes that *Anguillula stercoralis* is a separate and distinct parasite.

There are, however, certain points in which his observations vary materially from those of others. In the embryos found in the blood, and the smallest forms observed in the fæces, which were from 220 μ in length, by 10-12 μ in breadth, no internal structure could be made out. All recent observers, however, have noted that excepting perhaps, in the very earliest stages, the embryos developing from the eggs of both parasitic and free-living generations, show, already, the characteristic double œsophageal enlargement. Teissier's description of the adult parasite also varies from those given by Normand, Bavay, Grassi, Perroncito and others, in that he was able to distinguish but one spicule in the male, instead of two, as noted by all other authors. The character of the young larvæ, the presence of adult forms in the fresh dejecta, the slight differences in the structure of the male parasite, might give rise to the suspicion that Teissier may have been dealing with a different species of a closely allied parasite.

In a later article Teissier describes remarkable results obtained by inoculating these parasites into frogs—results which, if confirmed, would support this idea. After inoculation he believed that the worms developed in the intestinal tract and lungs of the frog, into giant forms. It is not impossible that these giant forms may have been *Ascaris nigrovenosa* [= *Rhabdonema nigrovenosum*].

It should be remembered, however, that Teissier is not the only observer who has recognized what he believed to be adult forms of *Anguillula stercoralis* in the fresh dejecta. Normand (1877) stated that he had met with all known forms of the worm at autopsy. In 1878 he asserted that "nothing is rarer than to see *Anguillula stercoralis* in a state of complete development in dejecta of recent origin"—a statement which would justify the inference that he had seen such forms. In this latter article he also expresses his positive opinion that the adult forms do develop in the gastro-intestinal tract.

In consideration of the fact that these views are at variance with those of most other observers, and of the somewhat indefinite character of Normand's statements, it has been assumed (Leuckart, 1883) that the sexually differentiated *A. stercoralis* may develop in the intestine, but only after death when the conditions are essentially the same as in cultures outside of the body, a theory which would account for one of Normand's statements. It must be acknowledged, however, that this does not cover his apparent assertion that he had met with adult forms in fresh dejecta.

In view of the statements of these two observers, it would, perhaps, at the present time, be unwise to deny the possibility that, in rare instances, the sexually differentiated intermediate generation may develop within the human host.

CLASSIFICATION.

The following classification has been generally adopted:

Family: *Angiostomidae.*
Genus: *Strongyloides* Grassi, 1879.
Syn.: *Pseudo-rhabditis* Perroncito, 1881.
Rhabdonema Leuckart, 1882, pro parte.
Species: *Strongyloides intestinalis* (Bavay, 1877).
Syn.: *Anguillula stercoralis* (Bavay, 1877).
Rhabditis stercoralis (Bavay, 1877).
A. intestinalis (Bavay, 1877).
Leptodera stercoralis (Bavay, 1877) Cobbold, 1879.
Leptodera intestinalis (Bavay, 1877) Cobbold, 1879.

Strongyloides intestinalis (Bavay, 1877) Grassi, 1879.
Pseudo-rhabditis stercoralis (Bavay, 1877) Perroncito, 1881.
Rhabdonema strongyloides Leuckart, 1883.
Rhabdonema intestinale (Bavay, 1877) R. Blanchard, 1885.

It may perhaps be well to emphasize one point in connection with the synonymy. Most of the recent text-books—Railliet (1895), Moniez (1896), Braun (1895), Weichselbaum (1898)—in their synonymy, refer to the special term *Strongyloides intestinalis* as having been introduced by Grassi in 1883. I have searched the literature with considerable care, but have been unable to find any reference to the worm under this name in Grassi's publications of that year. The name, however, was first used by Grassi four years before. In an article in the *Rendic. r. Ist. lomb.*, Milano, 1879, xii, ser. 2, p. 228, he proposes the generic name *Strongyloides*, and in a review of his own article, in *La med. contemp.*, Milano, 1879, iii, 495, he says: "He concludes by referring *Anguillula* to a new genus, *Strongyloides*, which" (*Anguillula*) "should therefore be called *Strongyloides intestinalis*." I have been unable to find any reference to this name in later publications by Grassi. In his articles appearing in 1883, he apparently accepted the classification of Leuckart—*Rhabdonema strongyloides*.

PATHOLOGICAL SIGNIFICANCE.

The fact that the worms were so frequently present in the severe diarrhœas of Cochin China led Normand (1876, 1877, 1878) to assume that they played an important part in the ætiology of the disease. At that time, of course, the so-called *Anguillula intestinalis* and *Anguillula stercoralis* were regarded as distinct species. Normand (1877) recognized the fact that the parasites may exist in the intestinal tract for considerable periods of time without producing any serious symptoms. Man may harbor the worm for years with little or no inconvenience; there may be noted, perhaps, only a slight softness of the dejecta, or occasional transient attacks of diarrhœa. Anything, however, which tends to diminish the resistance may offer to the worms the opportunity to produce those intestinal changes which result in the clinical picture of Cochin China diarrhœa. This view was upheld by Laveran (1877 [1, 2]), Davaine (1877), Dounon (1879), Roux (1877) and Ribeiro da Luz (1880). Libermann (1877 [1, 2]), however, was very reserved in his views as to the part played by the worm.

On the other hand, Chastang (1878), Breton (1879), Lutz (1885) and Calmette (1893) were inclined to doubt its pathogenicity. Grassi (1879 [1]) found the parasites in the stools of many healthy individuals

and while confessing that it was hard to believe that their action was not in some way harmful, yet he had never been able to make out any special symptoms attributable to the infection. He did not believe that the parasite was the cause of Cochin China diarrhœa. Later (1883 [2]) he asserts positively that "Anguillulæ are innocent commensals of man."

Golgi and Monti (1884, 1885), however, found distinct anatomical changes which they believed to be dependent upon the irritative influence of the worms, evidences such as could leave no doubt that, in some cases at least, the parasites must have a pathological significance, while Sonsino (1891) in a study of two fatal cases found evidence that the embryos may actually penetrate into the mucosa. He, as well as Golgi and Monti, frequently found worms occupying the lumina of Lieberkühn's ducts, while later, in the same cases, Venturi (Sonsino (1891)) discovered eggs and embryos in the depths of the villi and mucosa. Sonsino is strongly of the opinion that the worm may cause serious and even fatal changes. Riva (1891) likewise, from the study of a fatal case, is convinced of the pathogenic importance of the parasite, which he is inclined to consider the essential causal agent of the disease. The action of the worms is, he believes, wholly mechanical. More recently, Askanazy (1900) also has demonstrated that the worms may actually penetrate into the submucosa.

Teissier (1895 [1, 2]), indeed, has reported a remarkable instance in which small filariform larvæ were found in the circulating blood, worms corresponding closely to the larvæ of a parasite present in the stools, and identified by him as *Anguillula stercoralis*. As has been mentioned, however, there were peculiarities about the case observed by Teissier which are sufficient to give rise to some doubt as to the identity of the larvæ present in the blood.

Leichtenstern (1898), who has observed the constant presence, through years, of great numbers of the rhabditiform embryos in the stools of individuals in a relatively normal condition, while recognizing the fact that the presence of such enormous numbers of parasites may have a marked effect in increasing the severity of a diarrhœa when present, yet feels convinced that such a diarrhœa must owe its origin to some other primary cause, a view similar to that previously expressed by Calmette (1893).

Perroncito's (1880 [1, 2, 3, 6]) theory that the parasite played an equal part with *Uncinaria duodenalis* in the production of the severe anæmias observed in miners and tunnel workers, has long since been disproven. The removal of *Uncinaria* by proper treatment is sufficient to dispel

all symptoms of miners' disease, despite the fact that great numbers of the rhabditiform embryos of *Strongyloides intestinalis* frequently persist in the dejecta for long periods of time after the disappearance of all evidence of the sister worms.

The weight of evidence appears to be in favor of the view that, while the parasites may exist in the intestine for long periods of time without ill effects, they are by no means, as Grassi says, "innocent commensals of man." It would seem probable that this parasite alone may be the primary agent in many cases of chronic diarrhœa.

The deleterious influence of the worm is generally supposed to be purely mechanical [Golgi and Monti (1884, 1885), Sonsino (1891), Riva (1891), Askanazy (1900)], although Calmette (1893) suspects that the parasite may give rise to substances acting as chemical irritants.

CLINICAL MANIFESTATIONS.

The clinical picture described by Normand (1877) in his cases of Cochin China diarrhœa is that of a chronic diarrhœa, rather than of a dysentery. This commonly begins with mild dyspeptic symptoms, eructations, loss of appetite, etc., and a diarrhœa of moderate intensity, the stools being soft and pasty—three or four a day; the actions are often more frequent in the early morning hours. Not uncommonly, this condition is interrupted by temporary exacerbations; the attacks are sometimes dysenteric in character, the stools showing mucus and blood; in other instances more choleraic, the dejecta consisting of an abundant flux of a liquid yellowish material, while there may be vomiting, cyanosis and collapse. In many instances recovery occurs early in the course of the disease, the symptoms gradually clearing up. In other cases the patients pass on to a condition of extreme emaciation with great prostration. The anæmia is not, as a rule, very severe. Intercurrent dysentery is not uncommon and may terminate fatally.

Barbagallo (1897) and de Silvestri (1895) have reported cases in which certain nervous manifestations (headache, vertigo, tinnitus aurium, feeling of prostration) played a prominent part in the clinical picture. In de Silvestri's case there were no intestinal symptoms. All these manifestations ceased with the disappearance of the parasites under treatment with male fern.

TREATMENT.

The treatment, beyond those measures of rest and diet such as are applicable to all similar conditions, is not especially satisfactory. Normand (1877) fancied that the use of large quantities of olive oil was of a certain value purely from its mechanical action.

Perroncito (1881) believed that he had obtained results from large doses of the ethereal extract of male fern, doses as large as 12 grammes repeated daily until the disappearance of the parasite. In other instances he gave from 15-30 grammes in three doses during the morning, and repeated these daily, until the disappearance of the parasites from the stools. De Silvestri (1895) and Barbagallo (1897) in single cases, also report good results from the use of male fern. Perroncito insists particularly upon full and repeated doses, and believes that the failure to obtain good results depends upon the fact that observers have used insufficient quantities of the drug through too short a period of time.

On the other hand, Seifert (1883[2]) found that, in doses as large as 20 grammes, the ethereal extract of male fern was without effect. He obtained better results from large doses of thymol.

Grassi (1883[2]) also vigorously opposes the view that any known anthelmintic is of particular value in the treatment of this disease. This is based upon his own experience and upon the fact that adult parasites are hardly ever found in the stools even after the administration of large doses of the drugs.

Golgi and Monti (1884, 1885) and Riva (1891) also found that repeated and large doses of these anthelmintics appeared to have little or no result.

CASES.

The three cases which are here briefly reported are, I believe, the only instances which have been observed in the United States.

The first of these occurred in the Johns Hopkins Hospital nearly four years ago, and was recognized and studied by R. P. Strong, who was, at that time, a fourth-year medical student. Lieutenant Strong, who is now the director of the U. S. Army Pathological Laboratory

at Manila, has made a careful study of this case, which will be reported later by him, in the Johns Hopkins Hospital Reports.* He deserves entire credit for the discovery and recognition of the first instance of this disease noted in this country.

Case I.—*Amœbic dysentery. Intestinal infection with* Trichomonas intestinalis *and* Strongyloides intestinalis. *Abscess of the liver. Death. Autopsy.* K., a German tailor, aged 52, was admitted to the Johns Hopkins Hospital December 7, 1896, complaining of pain and swelling in the right side of the abdomen and over the lower ribs.

The *family history* was negative.

He had had measles in childhood, but had, otherwise, been a healthy man. He denied venereal disease. Up to six years before he had lived in Austria.

Three years before entry, he began to suffer from diarrhœa which has continued since that time. There were, daily, from two to five operations, which occasionally contained a little blood; these were never associated with pain. Three weeks before entry, he began to notice a painful swelling on the right side over the lower ribs. He had had no chills, and, so far as he knew, no fever. For two weeks before entry, there had been a slight cough without expectoration.

On *physical examination* the patient appeared emaciated and feeble. The heart and lungs were negative. The abdomen was somewhat distended; there was slight movable dulness in the flanks. On the right side, over the cartilages of the tenth and eleventh ribs, there was a large fluctuating swelling, 7½ cm. in diameter; no tenderness or redness. Between this and the spinal column there was another swelling, about 15 cm. in diameter, extending from the median line to the spinous processes, and from the twelfth rib over the crest of the ilium, downward for a distance of 7 cm. The skin over this was adherent, red and thin. The hepatic flatness was not increased.

Immediately after entrance this abscess was incised and evacuated. The cavity contained a large amount of thick puriform material with dark shreds and considerable greyish debris. The cavities extended into the liver. About one and a half pints of fluid were evacuated; this contained many large, actively motile amœbæ, showing the ordinary characteristics of *Amœba coli*.

The patient had daily, from one to four fluid, brownish, foul-smelling stools, which contained, besides *Amœba coli*, considerable numbers of *Trichomonas intestinalis*, as well as numerous small nematodes identified by Mr. Strong as the rhabditiform embryos of *Strongyloides intestinalis*.† The wound and the colon were irrigated with a solution of quinine, 1/1000.

The patient gradually failed and died on December 26.

*Since the completion of this article Strong's paper has been published. (See Bibliography.)

†The conclusions of Mr. Strong were confirmed by Dr. Charles Wardell Stiles of the U. S. Bureau of Animal Industry.

The protocol of the *autopsy* reads: "Amœbic dysentery; ulceration of the large intestine; retroperitoneal abscess; operation wounds; parenchymatous and fatty degeneration of the kidneys; fatty liver; fatty heart; intestinal infection with *Trichomonas intestinalis* and *Strongyloides intestinalis*."

"The abscess cavity measured 7 x 5 cm. on the surface of the liver and extended about 4 cm. into the substance of the organ. Scrapings from the wall showed numerous amœbæ." The rectum and colon are the seat of widespread ulceration. The ulceration, which, for the most part, is shallow, leads in the rectum, into an eroded, thickened, hæmorrhagic appearance. The ulcers vary in size; the largest are irregular in shape, measuring about 3 cm. in length by 2 in width. A typical ulcer of moderate size might be described as follows: "Shallow, smooth base, somewhat striated, evidently formed by the muscular coat of the intestine; white and shining, covered with whitish shreds. The outline of the ulcer is irregular but rather clean-cut, the edges are slightly elevated and are hæmorrhagic, neither shredded nor undermined." There are a large number of small pea-sized, and smaller, yellowish, slightly elevated areas, with red margins, which on careful study, appear as shallow ulcers, filled with a tenacious yellowish material. As they grow larger, they become like the typical one described above. About the middle of the transverse colon are two perforating ulcers, rather more hæmorrhagic about the edges; the mucous membrane is turned outward. Coverslips from the ulcers show active amœbæ. The small intestine is free from ulceration; here and there is a slight ecchymotic patch. The abscess of the liver communicates with the large retroperitoneal abscess opening outward as first described.

All along the tract Mr. Strong and Dr. Stiles found embryos of *Strongyloides intestinalis*. The parthenogenetic mother worms were found in the duodenum and jejunum. Careful studies by Mr. Strong resulted in the cultivation of but one adult male of the free living generation; adult females were never found. The direct transformation of the rhabditiform embryos into the filariform larvæ was apparently the rule. A similar observation has been previously made by Grassi and Segrè (1887), who found that, when the direct transformation predominated, the sexually differentiated forms found in the cultures were always males.

The point of origin of the infection in this case was not apparent. The disease may have been brought from Austria. The patient lived in the city, and drank city water.

Case 11.—*Chronic diarrhœa. Rhabditiform embryos of* Strongyloides intestinalis *in the dejecta. Great improvement.*

N. P., a boy seventeen years old, presented himself at the dispensary of the Johns Hopkins Hospital May 10, 1899, complaining of diarrhœa.

The *family history* was good. There was no history of pulmonary trouble or of hereditary disease. The grandmother, however, had suffered from diarrhœa for five years.

Personal History.—The patient had lived all his life in Richmond, Virginia, where he had been a newsboy. He had had measles, whooping cough and mumps as a child, and had suffered, off and on, from indefinite pains in both legs. Five years before entry, he had tertian ague. There was no history of pneumonia, typhoid fever, acute rheumatism, pleurisy, scarlet fever or chorea.

Present Illness.—Three years before entry, the patient began to suffer from diarrhœa, the attack coming on during the summer. The operations were very thin and yellowish in color, containing at times, a small amount of blood, though this had not been noticed for a year and a half. The passages occurred mainly at night, as many as seven or eight in twenty-four hours, but of late, only about four. There was, at first, considerable mucus in the stools, but this had been practically absent during the two years before entry. The diarrhœa had never been associated with pain. The patient, however, had grown gradually weaker, and during the second year of his illness, he had been compelled, on one occasion, to take to bed for two weeks. Four months before entry, he gave up work on account of weakness, though up to that time he had felt tolerably well.

The appetite was good; there were no dyspeptic symptoms.

Physical examination showed a slight, emaciated boy, of sallow color. The lips and mucous membranes were somewhat pale; tongue clean. The chest was long and narrow; the intercostal spaces, deep. Examination of the heart and lungs was negative. The abdomen was not distended. The spleen was not palpable. No peristaltic movements were visible. There was no enlargement of the liver; no glandular enlargements; no scars upon the shins. There was an irregular chloasma-like pigmentation over the cheeks, forehead and temples, and also over the lower thorax and lateral abdominal regions.

The *urine* showed no abnormalities.

The *blood* showed no marked leucocytosis; no apparent increase in the eosinophilic cells.

A rectal tube was introduced and a little, brownish, foul-smelling material was obtained which, besides muscle fibres, vegetable cells, granular debris and bacteria, contained a number of actively motile worms. In the fresh stools these measured all the way from 0.225 to 0.45 mm. in length by 0.02 to 0.03 mm. in breadth. They showed the characteristic structure of the rhabditiform embryos of *Strongyloides intestinalis*, and manifested a very active serpentine motion.

The worm diminished slightly in size toward the head, and gradually tapered down to a slender sharp-pointed tail. The periphery was somewhat refractive, while within, the substance was filled

with glistening, refractive, fat-like granules, which were rather larger toward the head than toward the tail. The digestive tract was clearly visible. The œsophagus, between one-third and one-fourth the length of the worm, showed a long bulb-like enlargement at the head, followed by a constriction, which was succeeded by a second, round or ovoid enlargement. The digestive canal was readily seen to pass through these enlargements, the anal outlet being situated at a distance equalling about one-tenth the length of the worm from the tip of the tail. The anterior lip of the anal outlet was slightly raised. The mouth of the worm appeared, as far as could be made out, to consist of a simple funnel-shaped depression. In some instances the lumen of the digestive canal appeared to pass, as a straight line, directly through the œsophageal enlargements. In others, a distinct triangular opening was to be seen in the middle of the second of the œsophageal enlargements; the outlines of this opening were glistening and refractive, indicating clearly the tridentate, chitinous armature described by other observers (Plate IX, Fig. B). In many of the active worms repeated and violent muscular contractions of the œsophagus were observed; these were especially marked about this tridentate opening, which appeared to open and shut with considerable force. The outlines of the cells bordering the digestive tract, of which, in some instances, a slight suggestion could be made out, were as a rule entirely hidden by the glistening granules above mentioned. A little below the middle of the worm, on the same side as the anal opening, was a small clear elliptical area—the rudiment of the sexual apparatus.

The boy refused to enter the hospital, and was observed in the out-patient department off and on up to June 21. As far as possible he was kept in bed, placed upon a liquid diet and given large doses of bismuth. On May 21 he was given santonin 0.25 (gr. IV) followed by castor oil, but no adult worms were found in the discharges. He was also given high rectal irrigations with a 1/2500 solution of quinine; on several occasions thymol was administered, a dose of 2. (gr. XXX) being given on successive hours, followed by a castor oil purge.

The diarrhœa gradually diminished and the stools became semi-solid in character, but still contained parasites.

No adult forms of the worm were ever found. The parasites always died a few hours after the stools were obtained, possibly owing to the fact that urine was mixed with the dejections.

On June 21, the patient went home, but returned to the hospital on July 7. He was immediately put to bed, and given a liquid diet.

The stools, at this period, were from three to four in number in the twenty-four hours. They were at first of a pea-soup consistency, having a peculiar, foul smell, and showing considerable numbers of the rhabditiform embryos.

For a time the only medicinal treatment consisted of large doses of bismuth.

The stools were collected, free from the urine, and various culture experiments were made. In some instances the fæces were placed in the thermostat and kept at body temperature. In others, they were kept near the thermostat at a temperature of about 30-35° C. Other specimens were kept at room temperature, 20-30° C. When but few parasites were present, the method suggested by Leichtenstern (1898) was followed. In the semi-solid or solid fæces an excavation was made into which a little water was poured. Some hours later considerable numbers of parasites were usually to be found in this fluid. The best results were obtained in those cultures made at a temperature of 30-35° C.

The smallest worms, found immediately after the passage, measured about 0.22 mm. in length. During the next several hours they were observed to grow considerably until the largest measured about 0.55. Within twelve hours the greater number of the worms in the cultures had lost the distinct œsophageal enlargements, and had become somewhat longer and more delicate in structure, measuring usually from 0.4 to 0.55 mm. in length and, in one recorded instance, as much as 0.7 mm. Sometimes examples were found measuring under 0.4 mm. The transverse measurement was from 0.016 to 0.022 mm. All trace of the rudiment of the sexual gland disappeared. The anal opening was not evident and the digestive tract was visible

only through about half the length of the worm. In the posterior half of the worm the granules were darker and apparently more abundant than in the anterior portion. Though the parasite, as a whole, was more delicate than the younger embryo, the tail, as shown in the drawing (Plate IX, Fig. C), was blunter, and more truncated.* The parasites became rather more active, showing most striking serpentine movements, the appearance coinciding entirely with that of the filariform larvæ of *Strongyloides intestinalis.*

Though the stools were carefully studied during the patient's entire stay in the hospital from July 7th to August 26th, and though cultures were made daily, no examples of the sexually differentiated, free living generation were found.

In many of the specimens, worms were seen in which the outer layer had the appearance of a refractive capsule, but no constant relation between this appearance and the development of the parasite was traced. The moulting or escape of the parasite from this capsule was not observed.

In one or two instances the worm was noted, under observation, to become suddenly motionless, while the capsule became shrunken and greatly wrinkled. The finer points in the internal structure of the worm were no longer to be made out.

Although, as has been noted, the stools were examined several times a day through a long period of time, only two eggs were observed. These were similar in appearance. Drawings and measurements were made of one seen on the twenty-fifth of May.

These eggs were of elliptical shape, with a thin, clear, yellowish shell, and granular contents which could be clearly seen to be in segmentation. The measurements were about 0.0675 by 0.0375 mm. The appearances have been admirably reproduced by Broedel in Plate IX, Fig. A.

The condition of the patient improved greatly during his stay in the hospital. During the greater part of the time, he received subnitrate of bismuth 1.3 grm. (grains XX) three times a day. On several occasions thy-

* The truncation of the tail was not noted in the detailed observations, which were made before a careful study of the literature was undertaken. In the admirable drawings, however, made from life by Broedel, this point is clearly brought out.

mol was given, in two doses of 2. (gr. XXX) each, and on July 28 the patient took two doses, separated by an interval of an hour, of 4. (℥i) each, of fluid extract of male fern, followed by a purge. Neither eggs nor mother worms were found in the stools. The stools gradually became more solid, though never formed; they diminished in frequency until, finally, there were only one or two in the twenty-four hours. The diet was gradually increased, and, on July 24, he was allowed the regular ward diet. On August 26 the patient was, at his own request, discharged; he had gained twenty-two and one-half pounds in weight. On discharge, however, the pasty stools still showed moderate numbers of the rhabditiform embryos. Through friends of the patient it has been learned that he has, since this time, regained his normal weight and strength. He has resumed his occupation and considers himself well.

Case III.—*Arterio-sclerosis. Chronic diarrhœa. Embryos of* Strongyloides intestinalis *in the dejecta. Secondary anæmia. Great improvement.*

J. S., a farmer, a native of Anne Arundel County, Maryland, was admitted to the Johns Hopkins Hospital July 19, 1900, complaining of diarrhœa and swelling of the feet.

Family history, good.

Personal History.—The patient had been a farmer all his life; his habits had been good; he had had no serious illnesses, excepting chills and fever fifteen years before entry. He had always lived in Anne Arundel County, having never been out of the State of Maryland, excepting for occasional visits to Washington.

Present Illness.—He had considered himself a healthy man up to six months ago when he began to suffer from diarrhœa and progressive weakness. The stools, very frequent and sometimes involuntary, were fluid but never contained blood. They were small in quantity. During two months before entry, the patient had, on several occasions, vomited a yellowish fluid. For two days he had had sharp, griping pains in the lower abdomen. For a month there had been œdema of the feet and legs.

There had been no increase in the quantity of urine, though there was increased frequency of micturition. During the last two months he had lost considerably in weight and much in strength.

The examination by Dr. Futcher, on January 20, showed a rather sparely nourished man with a sallow complexion; moderate thickening of the radial arteries; no arcus senilis. Physical examination of the lungs was negative. Heart sounds, feeble, but free from murmurs. The abdomen was slightly full, bulging in the flanks, everywhere flat excepting in the umbilical region, where there was tympany; hepatic flatness, continuous with the abdominal flatness.

On July 19, a count of the leucocytes showed 21,500 to the cubic millimetre. Rectal examination showed numerous external hæmorrhoids; the mucous membrane felt rather soft; the prostate was enlarged and firm.

The *dejecta* were fluid, containing considerable pus, great numbers of leucocytes, a few red blood corpuscles and a moderate number of characteristic rhabditiform embryos of *Strongyloides intestinalis*.

The *urine* showed, throughout, a rather low specific gravity, 1005-1015, a trace of albumin, and occasional hyaline and granular casts. The quantity was not accurately estimated.

The temperature was slightly elevated, 100.6°, on entry, but, with the exception of an occasional rise to a point between 99° and 100°, it remained sub-normal during the greater part of the patient's sojourn in the hospital.

On July 26, a blood count showed: Red blood corpuseles 3,560,000; hæmoglobin 57%.

On entry the patient was given a milk diet, and powders containing: Tannogen 0.65 grm. (grains X), bismuth. subnit. 2. (gr. XXX) every four hours; also diuretin, citrate of potassium and strychnine. On the following day, the medicines, with the exception of the strychnine, were omitted.

On the 24th, tannogen 0.65 (gr. X) every four hours, was again ordered, as well as diuretin 0.325 (gr. V) every four hours.

There was little improvement in the condition of the patient up to August 12, on which date he was given thymol 0.325 (gr. V) every four hours, which resulted in an apparent improvement for about two weeks, after which time the diarrhœa returned. On August 21 the tannogen was discontinued, and the patient was given subnitrate of bismuth 1.3 (gr. XX) every four hours. On August 31 the bismuth was omitted and the tannogen was discontinued again September 4, and salol 0.325 (gr. V) every four hours was ordered. On August 13, the thymol was discontinued and four doses of santonin were given at hourly intervals without any striking results. The number of movements, however, became somewhat reduced and the general condition of the patient was considerably better. There was a gain of twenty-one and a half pounds in weight between July 29 and September 17, the date of his discharge.

Cultures from the fæces, made according to the same methods as in the last case, showed the direct transformation of the rhabditiform embryos into the filariform larvæ; the change was complete, in many instances, within twelve hours. No adult forms of the sexually differentiated, free-living generation were observed. No eggs were found in the stools, nor were any examples observed of the parthenogenetic mother worm.

These three cases are interesting in that they are the first which have been observed in this country. Of especial importance is the question regarding their point of origin. In the first case it is possible, though scarcely probable, that the disease may have been acquired in Austria. In the second and third, however, the disease must have originated here.

The behavior of the parasite on culture was similar to that commonly observed in cases arising in temperate climates. In all three cases, the direct transformation of the rhabditiform embryos into the filariform larvæ predominated. In one instance only, was a single

sexually differentiated form of the intermediate, free generation observed.

Question might be raised with regard to the nature of the eggs observed in Case II. The size of the one in which measurements were made, exceeded that attributed to the eggs of the parthenogenetic mother worm by many authors. Thus, Grassi and Parona (1879) give the measurements of the eggs as 0.06 x 0.04 mm., while Braun (1895), in his manual, gives the measurements as 0.050 to 0.058 x 0.030 to 0.034 mm., and Railliet (1895) gives the same measurements. Again, most authors are unanimous in stating that the eggs are present in the stools only with the greatest rarity.

There can, however, be little doubt as to the nature of the two eggs observed in this case.

(1) In the first place it will be remembered that they were found only upon two occasions, although a careful daily search was made through several months. Moreover, all authors who have had much experience with the parasite, have noted the *occasional* presence of eggs in the fresh stools.

(2) Their general conformation agreed entirely with that of the eggs of the parthenogenetic mother worm.

(3) The measurements, though larger than those given by Grassi and Parona and in most text-books, agree, however, with those given by Golgi and Monti (1884, 1885) in their careful and accurate study, and also by Riva (1891). These observers state that the diameters vary from between 65 and 70 μ in length, by 30-39 μ in breadth.

Another objection which might be opposed to the assumption that these were eggs of *Strongyloides intestinalis*, is the fact that, in each instance, they were in the process of segmentation, and did not yet contain the completely developed embryo. Might they possibly have been the eggs of *Uncinaria duodenalis?* This can be easily ruled out, both on account of their greater size, as well as because of the fact that they were found on but two occasions. In uncinariasis the eggs are always numerous.

They could not have belonged to the sex-ripe intermediate generation: (1) because, on careful search, the adult parasites were never

found: (2) because they were found in fresh stools. It may not be impossible that these special examples may have represented eggs in which the process of development had, for some reason, been arrested.

Another question which arises in connection with this case is the following: Do these instances represent an outbreak of the disease due to parasites which have been imported within recent years, or are we to assume that this worm has long existed among us?

When one considers the infrequency with which systematic examinations of the fæces are made, it seems to me unnecessary to assume that the worm has been recently imported. It is highly desirable that, both in our hospitals and in private practice, more systematic and thorough examinations of the fæces should be undertaken than are at present customary. It is safe to say that if microscopical examination of the fæces were carried out as regularly and systematically as is the microscopical examination of the urine and the blood, a number of interesting and important observations would follow.

An especially interesting point in connection with the history of this parasite is its frequent association with *Uncinaria duodenalis*. Grassi (1879 [1]) early emphasized the frequency of combined infections, while Perroncito (1880 [1-6], 1881, 1882, 1883), Sahli (1882), and others have also brought out this fact in connection with their studies of the epidemic among the workers in the St. Gothard Tunnel. Of 30 of Grassi's autopsies in Milan in which *Anguillula intestinalis* was found, *Uncinaria* was also present in 25. Lutz (1885) in Brazil, in 35 cases of infection with this parasite, found a combined infection with *Uncinaria* in 30, or 85.7%. The conditions under which the two parasites flourish seem to be similar, and the discovery that one of the worms exists among us, should open our minds to the possibility of the presence of the other.* Especially should it emphasize the importance of a careful examination of the stools in all suspicious cases of anæmia.

* While this article is in press, Dr. John L. Yates, Assistant in Pathology, Johns Hopkins University, has discovered at autopsy a case of Uncinariasis (Anchylostomiasis) at the Baltimore City Asylum at Bay View.

The relation of the parasite to the diarrhœa in these three instances is somewhat questionable. In Case I, the exciting cause of the process is uncertain, the parasite having been associated with *Amœba coli*. In Cases II and III, however, there was no apparent cause for the diarrhœa other than the presence of these nematodes. And while, in both instances, the parasites were present upon the discharge of the patient, improvement was associated with a great reduction in their number.

In conclusion one may be justified in emphasizing the following points:

(1) Diarrhœa associated with the presence of *Strongyloides intestinalis* occurs in the United States.

(2) The observation, in the Johns Hopkins Hospital, of three cases within three years, cases originating probably in Maryland and Virginia, suggests that this parasite may be more frequent than has hitherto been supposed.

(3) As in most cases originating elsewhere, in temperate climates, the development of the sexually differentiated, free living generation was in these instances apparently unusual, the direct transformation of the rhabditiform embryos into filariform larvæ predominating.

(4) The discovery of the existence of *Strongyloides intestinalis* should emphasize the possibility that *Uncinaria duodenalis* may also occur in this country.

(5) More systematic examinations of the fæces both in public clinics and in private practice are much to be desired.

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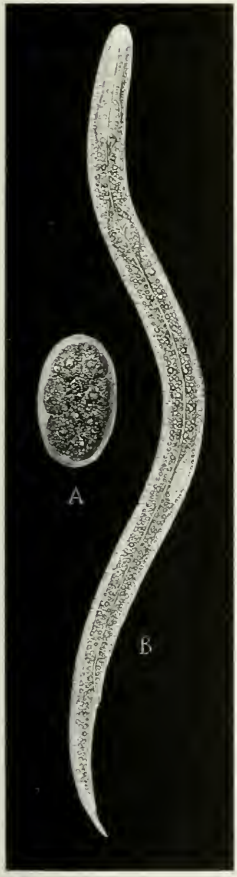
DESCRIPTION OF PLATE IX.

These figures were drawn from life by Max Broedel, Leitz, Objective 7, Ocular 3.

A. Egg of *Strongyloides intestinalis* (parasitic mother worm) found in stools of Case II, on May 25, 1899.

B. Rhabditiform embryo of *Strongyloides intestinalis* from the stools.

C. Filariform larva of *Strongyloides intestinalis* derived, by direct transformation, from a rhabditiform embryo.


A
B


C

THE EFFECTS OF SUBMINIMUM DOSES OF STRYCHNINE IN NEPHRECTOMIZED RABBITS.

By S. J. MELTZER, M. D., and W. SALANT, B. S., M. D.

(*A Study from the Rockefeller Institute for Medical Research.*[1])

By whatever method poison is introduced into the animal body, the general effect depends upon its presence within the circulating blood. The quantity present at any time is regulated by the degree of absorption of the poison into and its elimination from the blood. With a given dose of poison the effect is greater the quicker it reaches the circulation, or the more slowly it is eliminated from it. A dose which is ineffective when introduced into the alimentary canal, may produce a maximum effect when given by subcutaneous injection, or a dose which fails to produce a reaction in a single subcutaneous injection may, owing to better facilities for absorption, become distinctly effective, as was shown by Meltzer,[2] when divided into several—three or more—parts and injected into separate areas.

On the other hand, poor absorption may be compensated by restricted elimination; curare, for instance, is ineffective from the stomach. Claude Bernard and L. Hermann[3] have shown, however, that this was not the case when the ureters or the blood-vessels of the kidney are ligated. In a normal animal the degree of elimination of curare by the kidney is sufficient to keep the amount of poison within the blood below the effective minimum when its absorption is as slow as is that from the mucous membrane of the stomach. But even with this moderate absorption the amount of curare within the blood will sooner or later reach the effective dose

[1] This study was made in the Department of Pathology of the College of Physicians and Surgeons of Columbia University.

[2] Meltzer, *Jour. of Exp. Medicine*, 1901, v, p. 643.

[3] L. Hermann, *Arch. f. Anat., Physiol. u. wissensch. Med.*, 1867, p. 64.

as soon as there is no longer an eliminating power sufficient to prevent its accumulation.

These principles which were set forth by Claude Bernard, L. Hermann and others, and were recently pointed out again by E. Harnack[4] and other writers, have obviously their application also to poisoning by strychnine, the elimination of which takes place chiefly through the kidneys. M. Adam[5] discovered in a case of human poisoning the appearance of strychnine in the urine nine minutes after its injection, and Ipsen[6] states that in dogs it can be found in the urine five minutes after subcutaneous injection, and in rabbits even after two minutes.

Dragendorff[7] has shown that all the strychnine leaves the body unchanged, and Kratter[8] could, even with such a small medicinal dose as 2 mg., recover the entire quantity from the urine.

It seems, therefore, quite well established that the body of an ordinary animal, if it survives the administration, does not retain any strychnine, and nearly the entire elimination takes place through the kidney.

If the subcutaneous administration be accomplished very slowly, even more than a fatal dose may be injected without causing any characteristic effect, which is explained on the assumption that in this case enough strychnine is eliminated by the kidney to prevent the accumulation in the blood of an effective dose.[9] The reverse of this principle, i. e., that the restriction of elimination by the kidney will increase the effect of strychnine, has not yet, so far as we know, been seriously tested by experiment, but is apparently taken as self-evident. This at least is implied in an experimental argument by Carrara on the question of the ability of the animal tissues to render inert (entgiften) strychnine.

[4] E. Harnack, *Münch. med. Wochenschr.*, 1896, p. 1065.

[5] M. Adam, in T. and A. Husemann's Handbuch der Toxicologie, p. 510. Berlin, 1862-7.

[6] Ipsen, *Vierteljahrschr. f. gerichtl. Med.*, 1892, iv.

[7] Dragendorff, *Beiträge zur gerichtlichen Chemie*, iii, St. Petersburg, 1872.

[8] Kratter, *Wien. med. Wochenschr.*, 1882, pp. 214 et seq.

[9] Harnack, loc. cit.

von Czyhlarz and Donath,[10] who have made an investigation of the last mentioned question, believe that they have proven it in the affirmative by the following experiments:

The leg of a guinea-pig was tightly ligated and a fatal dose of strychnine was injected into the leg peripheral to the ligature. When the ligature was removed after a few hours, the animal survived without showing any effects of strychnine. The authors explained the result by the assumption that the tissues of the leg had fixed the poison.

In a series of experiments, Meltzer and Langmann[11] have shown that the results are positive only when minimum doses are used, and that in their opinion the most probable explanation of the fact is that this prolonged application of a tight ligature affects the absorbing capacity of the tissue of the leg to such a degree as to permit, after the removal of the constriction, of only very little absorption of strychnine at a time. This process is similar to that belonging to very slow injections, the continual elimination through the kidneys preventing the accumulation of strychnine in the blood to an effective dose. Against this interpretation, Carrara[12] brought forward an apparently very striking experiment. He repeated successfully the experiment of Czyhlarz and Donath on guinea-pigs, the kidneys of which were removed, and he argues that, if the absence of the effects of strychnine in the ligatured animals be due to slow absorption, the effect ought to appear in the nephrectomized animals in whom the strychnine cannot be eliminated, and consequently must accumulate in the blood to an effective dose. Here it is presupposed as a matter that is self-evident, that in a nephrectomized animal subminimum doses will soon aggregate within the blood to an effective dose, simply because an eliminating organ is removed.

It seemed to us, however, that this supposition, though very plausible and generally acknowledged, should not be accepted with-

[10] v. Czyhlarz and Donath, *Centralb. f. inn. Med.*, 1900, p. 321.

[11] Meltzer and Langmann, Ibid., 1900, p. 992, and *Medical News*, 1900, lxxvii, p. 685.

[12] Carrara, *Centralb. f. innere Med.*, 1901, No. 20.

out a direct test. Could it not be the case that after the removal of the kidneys other organs assume the task of elimination? This hypothesis is not new. Urea, for instance, is under normal conditions eliminated through the kidneys. We know, nevertheless, that in advanced destructive renal diseases urea is excreted through all kinds of organs, even through the lungs and the skin. Thus we have been led to make a series of experiments with the view of testing the validity of the principle in question, at least so far as strychnine is concerned. In other words, we have tried to ascertain whether repeated injections of subminimum doses, separated by shorter or longer intervals, become effective as soon as the sum total reaches an effective minimum.

Experiments were made on rabbits, which are very sensitive to strychnine. The subcutaneous method was employed exclusively. With regard to the effective doses, distinction is to be made between a fatal dose (dosis lethalis) and a dose which is only poisonous (dosis toxica)—tetanic attack with recovery. In nearly all of the experiments nitrate of strychnine was employed in a solution of 1 to 1000. We have made a few control experiments on normal rabbits. From these experiments, as well as from the very many data in the literature on this subject, it can be stated that 0.45 to 0.5 mg. are toxic doses, while 0.55 to 0.6 mg. are fatal doses of strychnine nitrate per kilo of rabbit in a single injection. Once in a while an exception is met with, when even such a single dose as 0.3 mg. per kilo produces a tetanus. But it hardly ever happens that 0.6 mg. per kilo fails to cause a fatal tetanus. The time between the injection and the outbreak of convulsions varies inversely as the dose; i. e., the larger the dose the shorter the interval; fifteen minutes is the average time for minimum doses, but it may vary from 4 to 40 minutes. In normal rabbits we have rarely seen a tetanus appear later than 30 minutes, and never after 40 minutes. In our experiments we relied on convulsions as the characteristic reaction; hyperæsthesia alone, especially in rabbits, is not characteristic enough to serve as a basis for comparison.

Knowing that in rabbits strychnine appears in the urine even a

few minutes after hypodermic injections (2 minutes, Ipsen), and knowing further that it takes from 10 to 15 minutes before a reaction sets in after a minimum dose, we may safely state that the quantity of the minimum dose of strychnine required to be present within the blood to call forth a reaction is equal to the amount absorbed minus the amount eliminated in the interval between injection and reaction. When we now remove the kidneys—the main path of elimination—we might expect that the minimum dose for the blood will be attained by the absorption of an amount which is perceptibly less than the normal amount of absorption; in other words, in nephrectomized animals we might expect to bring out a reaction with a smaller dose than is required in normal rabbits.

Our experiments did not confirm these expectations. The following is a table of some of the experiments with single injections in nephrectomized rabbits:

No. of exp.	Hours after nephrectomy.	Dose per kilo injected.	Results.
1	26	0.45 mg.	Slight short convulsion after 64 minutes; recovered.
2	1	0.5 mg.	Tetanus after 7 minutes; recovered.
3	3	0.5 mg.	No reaction.
4	23	0.5 mg.	Tetanus after 15 minutes; recovered.
5	24	0.55 mg.	Fatal tetanus after 15 minutes.

Comparing these results with the data above given for normal animals, we see that the required minimum doses in nephrectomized animals are not smaller than those for normal ones; on the contrary, there is a suggestion rather that the nephrectomized animals require a somewhat higher dose. However, the difference is too small and not constant enough to merit a further discussion. But it remains a noteworthy fact, that the removal of the kidney does not decrease the required amount of the minimum dose.

The most striking result, however, we obtained in experiments in which we employed subminimum doses repeated at shorter or longer intervals. Assuming that any dose of strychnine which cannot be eliminated by the kidneys remains in an effective state within the blood, which is the prevailing view, we might expect, no matter how small the doses and how long the intervals between the injections,

that, as soon as the sum total should reach the minimum of 0.45 or 0.5 mg. per kilo, there ought to occur immediately a tetanic outbreak, and that as there is no elimination, the tetanus ought to repeat itself indefinitely. Furthermore, it might appear that when the sum total reached the dose of 0.6 mg. per kilo, the first tetanic outbreak ought to terminate fatally. The results of our experiments, however, were entirely at variance with these expectations. On account of the importance of the results, we shall illustrate them by a number of protocols.

Exp. 6. Weight of rabbit, 1550 grm., double nephrectomy, Aug. 14, 1901, 12 M.

Aug. 14, 4.45 P. M., 0.3 mg. strychnine per kilo.
" 15, 12.45 P. M., 0.3 mg. " "
" 15, 4 P. M., 0.3 mg. " "
" 16, 1.30 P. M., 0.2 mg. " "
" 16, 5 P. M., 0.2 mg. " "

Rabbit died Aug. 17, at 10 A. M., 17 hours after the last injection, without having had convulsions. This rabbit then had received within 48 hours 1.3 mg. per kilo—that is, more than twice the fatal dose—without exhibiting any effects of strychnine poisoning.

Exp. 7. Rabbit, 1520 grm., double nephrectomy, Aug. 14, 12.30 P. M.

Aug. 14, 4.45 P. M., 0.3 mg. strychnine per kilo.
" 15, 12.45 P. M., 0.3 mg. " "
" 15, 4 P. M., 0.3 mg. " "
" 16, 1.45 P. M., 0.2 mg. " "
" 16, 4 P. M., 0.2 mg. " "
" 17, 9.30 A. M., 0.2 mg. " "

The animal was alive the same afternoon; was found dead the next morning. This rabbit received 1.5 mg. per kilo without any reaction.

Exp. 8. Weight of rabbit, 1900 grm.

Aug. 12, 1 P. M., double nephrectomy.
" 12, 4.30 P. M., 0.3 mg. per kilo.
" 13, 1 P. M., 0.3 mg. "
" 13, 5 P. M., 0.3 mg. "
" 13, 8 P. M., 0.2 mg. "
" 14, 12.30 A. M., 0.2 mg. "
" 14, 8 A. M., 0.2 mg. "

The rabbit died Aug. 15, at 10 A. M., or 26 hours after receiving the last dose of strychnine. This animal, too, had 1.5 mg. strychnine per kilo in the blood without showing any reaction.

Exp. 9. Weight of rabbit 1250 grm.

July 31, 3 P. M., double nephrectomy.

Aug.	1,	from	1	to	2 P. M.,	injected slowly, 0.43 mg.	per kilo.
"	1,	"	3	to	4 P. M.,	0.2 mg.	"
"	1,	"	5	to	6 P. M.,	0.2 mg.	"
"	1,	"	7	to	8 P. M.,	0.2 mg.	"
"	1,	"	9	to	10 P. M.,	0.25 mg.	"
"	2,	"	7	to	8 A. M.,	0.32 mg.	"

The rabbit died Aug. 3, at 8.30 A. M., or 24 hours after the last dose of strychnine had been injected, without any reaction. He received altogether 1.6 mg. per kilo of strychnine.

Exp. 10. Aug. 20, double nephrectomy, 4 P. M.

At 5.45 P. M. started giving hourly injections of 0.2 mg. per kilo until 11.50 P. M., altogether seven injections, 1.4 mg. per kilo, without any reaction.

Aug. 21 at 9.40 P. M. again hourly injections of 0.2 mg. per kilo, 20 minutes after the 3rd (10th) injection, convulsions occurred: the animal recovered soon, but died on the next day without having had convulsions again. This animal without kidneys received 1.4 mg. per kilo within seven hours without any reaction, and with more than 2 mg. within its body recovered easily and lived 24 hours longer without any strychnine symptoms.

Exp. 11. Rabbit, 1150 grm. Double nephrectomy finished at 11.30 P. M., Sept. 15, 1901. From 3 P. M. on hourly injections of 0.2 mg. per kilo, given five times; the last one at 9 P. M. The animal was observed for 2 hours longer; no reaction.

Sept. 16, 3 P. M., injected 0.3 mg. per kilo; no effect. 8.55 P. M., injected 0.4 mg. per kilo; 50 minutes later the animal had a few convulsions, recovered entirely after 20 minutes.

Sept. 18 at 7 A. M. injected 0.3 mg. per kilo without effect. At 2 P. M. again 0.3 mg. per kilo; no effect. Rabbit died on the morning of Sept. 19, without having had convulsions again.

Exp. 12. Rabbit, weight 2175 grm. Double nephrectomy, Sept. 14, 4 P. M. From 7 P. M. an injection hourly, 0.2 mg. per kilo; 10 minutes after the fourth injection a slight convulsion occurred; the animal recovered entirely in less than 2 minutes. Again injected at 1, 3, 5 and 8 P. M., 0.2 mg. per kilo without any response. Sept. 16 at 3 P. M.

injected 0.3 mg. per kilo with no effect. At 9 P. M. injected again 0.4 mg. per kilo; 35 minutes later tetanic manifestations occurred and the animal succumbed very soon.

We shall not, of course, report in detail all our numerous experiments. The few protocols which we have quoted are sufficient to indicate the correctness of the main fact which we wish to bring out here, namely, that rabbits without their main eliminating organs, the kidneys, can nevertheless tolerate the sum total of twice and thrice the fatal dose of strychnine without showing any reaction, if only care is taken to employ proper subminimum doses at not too short intervals. From a review of all our experiments we gather the following details: If the doses do not exceed 0.3 mg. per kilo at intervals of not less than four hours, injections can be made apparently indefinitely without causing any reaction. The same applies apparently also to doses of 0.2 mg. per kilo at intervals of about three hours; with larger doses or shorter intervals, the injections sooner or later induce a tetanus. How large a dose the sum total can attain before it becomes effective, varies with different animals. For instance, with a subminimum dose of 0.2 mg. per kilo in hourly injections, in some animals eight consecutive injections—with a sum total of 1.6 mg. per kilo—could be administered before a tetanus occurred or even marked hyperæsthesia was noticed. In other animals an effect appeared some time after the fourth injection, with a sum total of 0.8 mg. per kilo. However, in all cases the total exceeded the fatal minimum dose for the normal animal, and what is more, very rarely did any of these sum-total doses cause a fatal tetanus. If the injections were discontinued the animal recovered and survived the last injection for twenty-four hours or more without having another convulsion.

Leaving the statement and discussion of other details which we have observed in our present line of experimentation for some future occasion, our experiments brought out the following points:

1. For a rabbit without its chief eliminating organs, the kidneys, the minimum toxic and fatal doses of strychnine are, nevertheless, not smaller than those for the normal animals.

2. Even in a rabbit without its kidneys a single cumulative toxic dose of strychnine induces only one attack, or a few, and the animal soon recovers and shows no further effects of strychnine. Here a toxic dose remains apparently within the body without causing a continual effect.

3. If proper subminimum doses at proper intervals are employed nephrectomized rabbits can gradually receive thrice the fatal dose of strychnine without showing any reaction. Large fatal doses of strychnine are apparently accumulated within the body without causing any effect.

How are these remarkable facts to be interpreted? What becomes of the single toxic dose and of the cumulative fatal doses of strychnine within the body? Several explanations are possible. Thus it may be said that:

1. After the removal of the kidneys the act of elimination is carried on by other organs, for instance, by the gastro-intestinal canal. We know that secreting organs can substitute one another. We may then assume substitution also among excreting organs. An instance of this was mentioned above in the elimination of urea. It may be that even normally many organs are endowed with excretory mechanisms which have no opportunity to come into play so long as the great outlet through the kidneys, the excretory organs par excellence, is unimpaired. Collateral circulation after obstruction of the main artery transforms into large vessels fine capillaries which were previously hardly noticeable.

2. It is possible that strychnine is destroyed within the circulation by the blood, liver, etc. Before it was firmly established that strychnine is eliminated by the kidney, there were many theories as to the decomposition of strychnine within the blood. Lately the assertion was made anew by von Czyhlarz and Donath[13] and others, that blood cells, liver cells, and other tissues are capable of neutralizing strychnine. For the normal human body it has been established beyond doubt that none of the strychnine becomes decomposed by the tissues. However, it may be different in a nephrectomized animal. Falk[14]

[13] von Czyhlarz and Donath, *Ztschr. f. Heilkunde*, 1901.

[14] Falk, *Centralbl. f. med. Wiss.*, 1899, p 481.

has shown that in chickens, which are very resistant to strychnine, 90 per cent of the poison is decomposed by the tissues, and he suggests that this may be connected with the fact that there is very little liquid urinary secretion in these animals. This suggestion would hold good also for a nephrectomized animal.

3. It is further possible that within the blood of nephrectomized animals substances develop which do not decompose strychnine, but neutralize its effect upon the nervous system. Uræmic coma might be mentioned in this connection, indicating that there are indeed in the blood of animals with impaired kidneys substances which affect the functions of some nerve cells.

4. It is also possible that in nephrectomized animals absorption from the subcutaneous tissues is impaired on account of the increased blood pressure. This, however, would not explain why a toxic dose, already within the blood, does not continue to be effective. Besides, we found that a single minimum dose for a normal animal is also effective for the nephrectomized animal, which shows that absorption cannot be impaired, so that non-absorption cannot be the chief reason for the failure of the large doses of strychnine in cumulative administrations to cause some reaction.

All these hypotheses are open to experimental study which will be carried out at some future time.

We started out to test the generally acknowledged assumption, implied in the argument of Carrara, that when the kidneys, the chief eliminating organs for strychnine, are removed, subminimum doses will be effective as soon as the sum total reaches the effective toxic or fatal dose. We have shown that this assumption is incorrect, and that even thrice the fatal dose can be gradually introduced without producing any effect.

The experiments teach us also a general lesson in experimental science. Because we know that a certain organ has a certain function, it is commonly assumed that we abolish the function by removing the organ. We have seen that this is not true. Elimination is the preventive of cumulation. After removal of the eliminating organ, it might have been expected that the cumulative effect would

remain unrestricted. We have found that cumulation was nevertheless manifested to only a slight extent. Then again, if an organ is removed and a function persists, it has been assumed that normally the function does not belong to this organ. In nephrectomized animals, cumulation is very little manifested; nevertheless, we know it is the eliminating kidney which controls cumulation in normal animals. This last remark applies to a statement made by Leube in connection with our subject. Leube[15] in attempting to establish (34 years ago) the path by which strychnine is eliminated, thought of the kidney, and tied the ureters or the blood vessels, in the expectation that a small dose, which causes a mild effect in a normal animal, would have violent results in the operated one. Leube was disappointed in his results. Not only was the effect not stronger, but in one animal a certain dose, which two days previous to the operation caused a mild effect, after the operation had no effect at all. Leube explained this case as an instance of adaptation, and gave up the kidneys as the eliminating organs. We know that there is no adaptation of the body to strychnine (Hare[16] and others). We also know now that the kidney is the eliminating organ for strychnine in normal animals, and Leube's error in his conclusion is just the one to which we referred above. What Leube found is the same that we have seen in our experiments, namely, that the minimum dose for nephrectomized animals is the same as for normal ones, if not even a trifle higher.

Our results have also direct practical bearings. It has been argued by physiologists and pharmacologists (L. Hermann for instance) and has been repeatedly maintained by clinicians, that in chronic diseases of the kidneys, when the eliminating power of this organ is considerably reduced, great care should be exercised in the administration of poisonous medicines, lest they may accumulate in the blood with fatal effects. According to our experiments with strychnine on animals entirely without kidneys, fatal doses may be gradually introduced without effect, and there is a great difference between even a

[15] Leube, *Arch. f. Anat., Physiol. u. wissensch. Med.*, 1867, p. 629.

[16] Hare, *Amer. Jour. of Physiology*, 1901, v, p. 333.

maximum medicinal dose and a minimum toxic dose. The animal body apparently possesses a mechanism capable of regulating the cumulative capacities of the blood even in the absence of the kidneys. The influence of removal of the kidneys on the cumulative effect of other poisonous substances has not yet been studied. Thus the fear of cumulative effect in renal disease rests at present apparently on theoretical grounds alone.

CONTRIBUTION TO THE PATHOLOGICAL ANATOMY OF MALARIAL FEVER.

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PLATES X–XV.

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INTRODUCTION.

As with many other infectious diseases, the pathological anatomy of malarial fever had been thoroughly studied long before the discovery of the parasitic cause of the disease. Of the early studies it may be said, from the standpoint of our present knowledge, that they had nearly exhausted the subject of the more evident pathological changes in the viscera, and were essential, indeed, to the final discovery of the parasite, but that in the absence of a knowledge of *Plasmodium malariæ* their numerous misconceptions greatly detract from their value for present purposes. Of current writers,

Laveran is probably most familiar with the value of these studies, and in the last edition of his work properly honors them with abundant reference. Since the discovery of the parasite the entire field has been extensively reviewed, principally in Italy and France, and to some extent in America, Germany and England.

Nevertheless the records of close microscopic study of the lesions of ordinary malarial infection are by no means excessively numerous; much yet remains to be learned, possibly from a microscopic study of the visceral lesions, concerning the parasitology of the disease, while its numerous irregular forms still offer one of the most inviting fields for pathological research. An increased value still attaches to such records from the fact that malaria is seldom fatal in localities where a full clinical history can be supplemented by a complete microscopic examination of the viscera.

Considerations of this nature seem to justify the detailed character of the present contribution, which consists of the available clinical histories and the records of the gross and microscopic changes in the viscera of several cases of malaria, together with a discussion of any important features that the cases may present and their bearing on previous studies.

Most of the material was collected at Camp Wikoff, Montauk Point, Long Island, and the remainder was secured at Roosevelt Hospital or elsewhere in New York City. For the clinical records and notes of some autopsies of the Montauk cases, I am greatly indebted to Dr. Delafield of New York, and to Drs. Cotton, Allen, and Mosher of Boston. Case I, at Roosevelt Hospital, was under the care of Dr. W. H. Thomson, who kindly placed the clinical records at my disposal. Dr. Eugene Hodenpyl and Dr. John H. Larkin have each kindly contributed material and autopsy notes of one case.

PART I.

REPORTS OF CASES.

CASE I.—*Estivo-Autumnal Malaria. Sub-Acute Course. Blood Containing only Crescents for two weeks before death. Prolonged Coma. Absence of Parasites in the Brain. Peculiar Deposits of Pigment in Renal Epithelium.*

F. H., 64 years. No important previous illness. Spent some time in Long Island City, N. Y., shortly before illness, which developed in New York City.

September 25, had a chill followed by fever and sweat. Chills recurred every other day till October 2-3, when they became irregular and less marked. Admitted to Roosevelt Hospital, service of Dr. W. H. Thomson, Oct. 6, 1896. Diagnosis, malaria. Treatment, quinine and ginger, aa. grs. XXV-XL daily. Arsenic later, Fowler's solution, gt. XV. daily.

The patient seemed to improve slightly, the temperature falling gradually, reaching 99° F. on Oct. 10, and remaining at about that point until Oct. 20. There was from the first marked insomnia and tendency toward mild delirium at night, partly controlled by sedatives, until Oct. 11, when the delirium increased, and periods of mild coma supervened. About Oct. 16, the coma deepened and became continuous till death. There were no evidences of uræmia, and the coma was clearly of the malarial type. There was one slight paroxysm of fever on Oct. 20-21 (101.4°), and on the 23rd the temperature began to rise steadily, reaching 108° on the 25th, just before death.

The *urine* was passed in considerable quantity, of acid reaction, 1020 sp. g., and in the last two weeks of the disease contained a trace of albumin and a few hyaline and granular casts. No mention was made of pigment in the urine.

I had no opportunity to examine the *blood* until Oct. 12, when it was found to contain an enormous number of spheroidal, ovoidal, and young crescentic bodies, as many as ten appearing in one field of the immersion lens. Prolonged and repeated search (4-5 hours) failed to show any rings. There were the changes of secondary anæmia with marked loss of Hb. and moderate variation in the size of the red cells. The leucocytes were slightly reduced in numbers. Mononuclears 35%; polynuclears 60%; eosins 5%. A few richly pigmented mononuclear cells were seen. Oct. 13. The parasites were as numerous as before but there were now some elongated and apparently full grown crescents, while the spheroidal bodies were less numerous. No rings seen. Oct. 15. The adult crescents now outnumbered the smaller forms, which were however still rather abundant. No rings could be found. The anæmia appeared rather more pronounced. There was no leucocytosis, but the eosins were still increased. Oct. 21. The numbers of parasites were still large and the forms were about equally divided among elliptical bodies and adult crescents. Oct. 25. Eight hours before death the blood was found to contain very few parasites. In the course of this search 8-10 young crescents and spheroidal bodies were encountered, but no rings. There was a moderate polynuclear leucocytosis.

Autopsy.—12 hours after death. The anæmia appeared slight. No œdema. No jaundice. *Lungs*, moderately congested and œdematous. *Spleen*, slightly enlarged, very soft, of characteristic slate color. *Liver*, slightly enlarged, of characteristic slate color. *Stomach and intestine*, negative. *Serous membranes*, slightly discolored in places. *Kidneys*, size about normal; capsules adherent in places, elsewhere surface is smooth; cortex slightly irregular; markings distorted in places. The cortex is light red in color, the medulla and papillæ very dark red, or rusty. The *marrow* of ribs and vertebræ is hyperæmic, and of slight chocolate tinge. *Brain*, moderately œdematous; not discolored; shows no petechiæ; the basal vessels appear normal.

Microscopic Examination.—Liver.—There is a very extensive deposit of pigment, in the form of discrete and conglomerate grains and masses, in the endothelial cells, macrophages, and occasionally in the liver cells. Much of this pigment is very compact and dark, and is evidently of rather old formation. The numbers of pigmented spheroidal or ovoid bodies are considerable, and many of these lie free in the capillaries, but all stages of the ingestion and destruction of parasites by phagocytic cells can be followed. Some peculiar bodies, possibly derived from malarial organisms, are to be seen. One of those rather frequently seen is spheroidal, a little larger than a red cell, with a thick shiny hyaline outer border, and showing a central mass of yellowish pigment granules. In some of the larger vessels there are several spheroidal bodies twice as large as a red cell, hyaline and faintly bluish stained throughout, and exhibiting a moderate number of central pigment granules. A good many small pigmented parasites were seen.

Spleen.—The deposit of pigment is extreme. Much of this is old pigment in compact masses within phagocytes, but there is a considerable number of free pigmented parasites, and all stages of their ingestion and destruction can be seen. Neither rings nor rosettes are seen. There is the usual cellular hyperplasia of the pulp cords.

Marrow.—The marrow of the ribs and vertebræ contains a moderately rich deposit of pigment which is usually limited to the phagocytes. Very little pigment is seen in the vessels, and very few parasites were identified either in smears or sections. The eosinophile cells and giant cells are increased. The nucleated red cells are abundant and some are slightly increased in size.

Kidney.—There is a moderate chronic diffuse nephritis with growth of new connective tissue in small wedge-shaped masses in the cortex. The glomeruli are apparently normal. The tubules show swelling of the lining cells, and in a few places the cells of the convoluted tubules are necrotic. They all contain much granular yellowish pigment giving the reaction of hæmosiderin. Very few parasites could be identified. The deposit of pigment, however, is very abundant and of peculiar distribution. The glomeruli contain more than the usual number of pigmented cells of the usual character. The larger vessels of the cortex are sometimes injected with blood in which are considerable deposits of brown granular or crystalline pigment. In the neighborhood of these vessels there are often many clumps of similar pigment and these are sometimes found in the lining cells of the convoluted tubules which are elsewhere entirely free from brown pigment. The limitation of this pigment in the cortex to the vicinity of vessels, strongly indicates that the crystalline deposits have resulted from the diffusion of dissolved Hb. or of escaped red cells.

Throughout Henle's loops, and especially in the ascending arms and in the collecting and discharging tubules the number of clumps of pigment is enormous. The vessels here, while injected with blood, are nearly free from pigment, which lies exclusively *in the lining epithelial cells of* the tubules, some cells containing 40-50, or more, clumps in a single section (Plate XI. Fig. 9). These cells, moreover, fail to show marked evidences of granular or fatty degeneration, or of fragmentation, but their protoplasm is uniformly finely granular, their edges are unbroken, and their nu-

clei unchanged. On close inspection these pigment clumps are found to be composed usually of small blunt-pointed crystals, sometimes of granules, of dark brown color, arranged usually in a rather compact circle or sphere. Some of the pigment is more diffuse. Occasionally a clear space surrounds the pigment clump, but the great majority of the pigment circles lie bare in the cell.

Brain.—Throughout the medulla, cerebrum, and cerebellum, the vessels are nearly free from pigment and parasites. In some sections from the frontal cortex there are a few pigmented endothelial cells and an occasional pigmented parasite, but most capillaries, though considerably injected, are free from all traces of parasites or their derivatives. In many of the pericellular lymph spaces throughout the cortex there were peculiar structures, the nature of which I have been unable to determine. These bodies consisted mostly of elongated fibrils or rods with tapering ends, about 0.5 to 1 μ in thickness, and 5-15 μ in length. They were sometimes single, more often multiple, and arranged in rosettes, or spirals, or in concentric layers, or irregularly clumped. They stained densely with methylene blue, faintly with hæmatoxylin. Similar deposits were found in other cases of malaria, and in one case of tuberculous meningitis. They may for the present be classed with the artifacts of nervous tissue.

Epicritical:—In the above somewhat anomalous case there are several features of interest. The development of a case of fatal malaria in a patient who for twenty-five years had not been away from the vicinity of New York City is unusual. While autopsies on cases of malaria are not extremely rare in this locality, they are usually cases which were infected in southern latitudes.

While quinine in moderately large doses, with arsenic, controlled the active sporulation of the parasite and reduced the temperature, the treatment failed as usual to have any effect upon the crescentic forms, which persisted in enormous numbers until, rather suddenly in the last few days of the disease, they disappeared almost entirely, although the patient died in hyperpyrexia. This pyrexia is no indication of a failure of quinine to control the infection, as none of the young forms were seen after Oct. 12, and the terminal fever must be referred to other causes.

The prolonged delirium and coma are the chief clinical features of the case. There seemed little ground for doubting that the mental condition was referable to the malarial infection, because the coma was established before the urine contained casts and albumin; the changes in the urine were never marked; there were none of the usual concomitant signs of chronic uræmia, such as œdema, muscular twitchings, etc. The general condition of the patient was typically that of malaria; microscopic evidences of extreme malarial infection were

found in the blood, liver, spleen, marrow, and kidneys, while the evidences of nephritis were very much less marked than those usually found in cases dying in chronic uræmia. Neither can the coma be referred to the presence of organisms in the cerebral vessels, as none were found there, and it becomes necessary to regard the cerebral symptoms as dependent upon other conditions, probably toxic, associated with the severe malarial infection. This conclusion is in accord with the evidence furnished by other cases of the present series, which fails to support the view that malarial coma is always dependent on the presence of parasites or embolic processes in the cerebral vessels. (See especially Case VII.)

The most striking pathological feature of the case is the massing of pigment in the kidneys, especially in the cells of Henle's loops. A careful review of the microscopic studies of the viscera in malarial infection, which is believed to be fairly complete, fails to show the report of any similar condition in uncomplicated malarial fever. In the studies of Bignami,[1] Guarnieri,[2] Marchiafava and Celli (1887),[3] Kelsch and Kiener,[4] Stieda,[5] Bastianelli (1894),[6] Barker,[7] Benvenuti, Thin,[8] and others, the number of parasites and deposit of pigment in the kidneys are described as moderate. This general rule is explained by Bignami by the rapid renal circulation. The condition in the present case appears to resemble that found in the kidneys in hæmoglobinuric malarial fever, in some cases of which large deposits of pigment have been found, but differs from them in the peculiar distribution of the pigment, and in the absence of hæmaturia.

On first observing the pigment deposits in the epithelial cells I was inclined to regard them as the remnants of recent parasites, on account of the close resemblance of many of the pigment circles to those of the crescentic bodies, and from the presence of vacuoles about some clumps. Further observations on this and other cases, notably Case VII, led to the conclusion, however, that most of such pigment wreaths here and elsewhere have never been associated with parasites, but are derived from altered red cells. In the present instance this conclusion

[1] *Atti d. R. Accad. med. di Roma*, 1890; 1893.
[2] Ibid., 1887.
[3] Ibid., 1887.
[4] Maladies des pays chauds, Paris, 1889.
[5] *Centralbl. f. allg. Path. u. path. Anat.*, 1893, iv, p. 321.
[6] *Bull. d. R. Accad. med. di Roma*, 1893; 1894.
[7] *Johns Hopkins Hosp. Rep.*, 1895, v.
[8] *Lancet*, 1896, i, p. 1414.

is based upon the absence of any trace of the body of a parasite about the pigment clumps, the abundance of the clumps about the injected vessels of the medulla, and the crystalline form and peculiar arrangement of the grains of pigment in many of the clumps.

Case II.—*Æstivo-Autumnal Malaria. Extreme Malarial Infection. Very large numbers of Parasites in Viscera, especially in the Heart Muscle. Rosettes in the Peripheral Blood. Extensive Pigmentation of Serous Membranes.*

J. B., 40 years. Went to Santiago in June, 1898, and remained about five weeks. During the last two weeks he felt feverish in the afternoon and complained of abdominal pain and diarrhœa. Never had a chill. The same symptoms continued during four weeks after his return to New York, when he took no medicine. Lately his chief complaints have been weakness and increasing pallor, but he has noticed no recent paroxysms. On August 15, he felt much worse and walked to the hospital with difficulty. When first seen he was apparently moribund but could answer a few questions as above. He soon became comatose. The pulse was very feeble, 124 per minute; temperature 101.6°; respiration 40. Extreme pallor and slight jaundice were noted. On physical examination the heart sounds were very feeble. The spleen was not palpable but the area of splenic dullness was enlarged. The *urine* was alkaline, sp. g. 1014; it contained a little albumin, but no casts were seen. During the night he vomited occasionally; delirium alternated with stupor; the pulse failed steadily; the temperature rose to 103.8° F., and he died 14 hours after admission.

Autopsy.—10 hours after death. *Externally*, extreme pallor, slight jaundice, no œdema. Subcutaneous tissues show a peculiar brownish yellow discoloration. *Lungs*, moderately congested and œdematous. *Heart*, size normal; muscle very flabby and pale; valves normal; chambers slightly dilated. *Liver*, surface and section of dark slaty color; outlines of lobules indistinct; size and consistence normal. *Spleen*, 1½ lbs., uniformly enlarged, of very dark chocolate color, pulp diffluent. *Kidneys* show signs of acute degeneration. *Gastro-intestinal tract* shows throughout a moderate catarrhal inflammation, but no hæmorrhages or erosions. *Serous membranes* all show small areas of black pigmentation. Peritoneum is of a dark slaty color and in the parietal pleura there are some large irregular patches of dense black pigmentation. Marrow of ribs and vertebræ is very hyperæmic and of dark chocolate brown color. The *blood* is very anæmic and of distinct chocolate tinge. The *brain* could not be examined.

Microscopic Examination.—Blood.—Smears taken on admission showed a large number of æstivo-autumnal parasites. Most were of the signet-ring form, without pigment, and all except the earliest stages were about equally represented. In a few of the larger rings one or two fine pigment grains were noted. Besides the rings there were a good many older forms with clumps of pigment. A few rosettes were found in the blood. The red cells showed the changes of a severe secondary chlorotic anæmia.

Heart Muscle.—The number of parasites in the red cells filling most of the capillaries is enormous. At many points nearly every red cell contains one or more organisms and the vessel appears to be distended with masses of infected red cells and pigmented leucocytes (Plate X, Fig. 7).

The forms of the parasites are about equally divided between non-pigmented rings, pigmented spheroidal bodies, and rosettes; but no crescents or distinct ovoids could be found. There are in the vessels a few pigmented endothelial cells of the usual appearance, and many richly pigmented leucocytes.

Most of the muscle cells contain a moderate deposit of large greenish yellow granules. There are no evidences of fatty or other form of degeneration of the muscle cells.

Lungs contain a great many richly pigmented phagocytes with parasites in all stages of degeneration. The capillaries are frequently distended and sometimes apparently occluded by masses of red cells, pigmented endothelial cells, leucocytes, and macrophages. The vesicles contain a few large pigmented cells. Parasites are not more numerous than in the peripheral blood.

Kidney.—The capillaries everywhere contain a moderate number of red cells harboring parasites. Most of these are the non-pigmented, ring-shaped organisms. A great many richly pigmented spheroidal parasites were seen in the same situation as the rings, and a few segmenting bodies were noted. In no place were the capillaries distended or occluded by masses of infected red cells and pigmented leucocytes, as was noted in the heart muscle. Evidences of phagocytosis are moderately abundant and extremely distinct, especially in the glomeruli. The phagocytic cells are always either mononuclear leucocytes or endothelial cells, the tubule cells being free from pigment. The observed phases of the process included the inclosure of an entire red cell infected with an unpigmented ring; the inclosure of one or many pigmented spheroidal bodies; all stages of the destruction of the bodies of inclosed parasites; and finally the circles and clumps of fine pigment grains, which remain after the destruction of parasites and red cells. From one to twenty circles were counted in large macrophages lying free in the capillaries. The glomeruli and the capillaries of the medulla contain more pigmented cells and parasites than do the cortical vessels. There is a marked acute degeneration of the lining cells of the convoluted tubules, but no signs of chronic nephritis. The tubules are slightly dilated, and contain either casts, or coagulum, or fragments of epithelial cells.

Marrow.—The deposit of pigment is very marked but much less than in the liver and spleen. The pigment is found in the endothelial cells, and especially in the large and giant mononuclear cells of the pulp cords. In these cells all stages of the ingestion and destruction of parasites could be followed. The number of parasites within red cells is considerable and larger than in the liver and spleen.

In sections of ribs and vertebral bodies very few fat cells are present, the marrow being excessively cellular. Nucleated red cells are abundant and some are of slightly increased size. The eosins and giant cells are distinctly increased.

In smears of the expressed marrow the same conditions were seen distinctly. Minute intracellular rings are very abundant, many cells containing two, and some three, or four, such parasites. Twin parasites were found in the cells, also, in the form of large vesicular bodies, one-third the diameter of the cell, and sometimes showing one or two very minute

pigment grains, but beyond this size, no twin parasites could be found. Well pigmented parasites were always single, and no cell was found containing two well developed spheroidal bodies.

A few minute rings and a great many pigmented spheroidal bodies were found apparently extracellular, a position which I am inclined to refer principally to post-mortem and artificial processes. Rosettes were abundant, and in several which were considerably flattened 18 spores could be accurately counted. A moderate number of young and middle-sized crescents were seen.

The phases of phagocytosis were quite distinct. The great majority of pigmented cells, and the only ones containing excessive pigment deposits, were the large mononuclear cells of the pulp cords. The pigment was found in the form of discrete grains, or often in compact clumps, or circles. From one to thirty or more of these pigment-clumps were seen in the larger cells. In many instances the circle of pigment surrounded a vacuole somewhat larger than the body of a parasite. In heavily pigmented cells irregular masses of granular pigment were usually found in the perinuclear region and elsewhere. The parasites seen within phagocytes included a few non-pigmented rings and many faintly staining pigmented spheroidal bodies. Many stages of the degeneration of parasites were seen in the bodies of these cells.

The frequent appearance of a circle of pigment grains around a small vacuole is probably referable to the complete destruction of the red cell (Plate X, Figs. 1 and 4). Many of these large circles of pigment grains surrounded a central compact clump, as though the englobed cell had contained a pigmented parasite. A great many small spherical masses of fine pigment grains, identical in appearance with the pigment clumps of full grown parasites or rosettes, were seen, and traces of the bodies of the parasites were often detected about the clumps. Many discrete coarser grains and clumps without vacuoles or surrounding granules seemed to represent that pigment which had been absorbed from the plasma after its discharge from the parasite. Larger conglomerate masses of older pigment were abundant, but even in these masses a granular structure was usually visible.

Finally, in the marrow smears, the formation of peculiar *vacuolated leucocytes* could be traced from the smaller and medium-sized mononuclear cells of the marrow. In these cells the nucleus gradually lost its affinity for methylene blue and became indistinguishable from the cell-body. At the same time, in the nucleus and cell-body, hyaline globules developed and gradually increased in size until the entire cell was reduced to a coarsely reticulated faintly staining mass, identical in appearance with the vacuolated leucocytes seen in the circulation (Plate XI, Fig. 8). This process affected both pigmented and non-pigmented cells.

On the other hand no resemblance could be traced between any of the stages of these degenerating cells and the various forms of parasites to be found in the marrow, none of which, when admitting of positive identification in stained specimens, exhibited similar degenerative changes. *In the fresh marrow juice these leucocytes very closely resembled the forms of large vacuolated parasites described by some writers.*

Spleen.—The black color of the spleen is found to be referable to very heavy deposits of pigment, lying in endothelial cells, large mononuclear cells of the pulp and sinuses, and leucocytes in the sinuses, and in parasites infecting red cells. The outlines of the sinuses are no longer visible, being choked by the influx of pigmented cells, or distended or ruptured thereby. The Malpighian bodies are much reduced in size. No foci of large cells free from pigment were to be seen in the pulp tissue.

The parasites are very numerous and of all forms except crescents, but the majority appear to be inclosed in various phagocytes.

Liver.—The pigment deposits are extremely rich and are found in the long swollen endothelial cells of the capillaries and in large mononuclear cells lying often apparently free in the capillaries, and some of which are probably leucocytes. The parenchyma cells are free from pigment. The number of parasites is very large. Most of them are inclosed within the above phagocytic cells, in which all stages of their degeneration are to be seen (Plate X, Figs. 1 and 2).

In the red cells a moderate number of rings, pigmented spheroidal bodies, and a few rosettes are seen. In the capillaries of Glisson's capsule the numbers of parasites within red cells are very much greater than in the intralobular capillaries. The chromatic network of the liver cells stains faintly with methylene blue and is very ragged and irregular. There are no signs of fatty degeneration. The cells usually contain a deposit of large greenish granules.

Epicritical:—Among the features of this case, attention may be drawn to the rather sudden termination after a period of comparative freedom from severe symptoms, in which respect there is nothing very anomalous; to the prominence of the cardiac symptoms; to the acute degeneration of the kidneys; and to the activity and extent of the phagocytic process. These features may best be considered in connection with the pathological conditions found in the viscera.

As the patient's condition had not led him to seek medical assistance until a few hours before his death, it must be supposed that the paroxysms immediately preceding the fatal attack were mild, as is also indicated by the further history obtained. Such pronounced heart weakness occurring early in the first severe paroxysm of the relapse would seem to class the case with those of acute cardiac failure in pernicious malaria. It was noted in the physical examination on entrance that the first sound of the heart was barely audible, and the pulse extremely feeble. The microscopic examination showed the presence of unusually large numbers of parasites in the visceral capillaries, the heart muscle containing an excessive proportion of them. All the small vessels in this locality were filled or distended with blood containing a colossal number of parasites in all stages of development, with an abundant admixture of pigmented leucocytes. A considerable

mechanical effect of such a massing of parasites in obstructing the circulation can hardly be doubted, although no signs of complete thrombosis of small vessels could be found. On the other hand, no inflammatory reaction in or about the vessels or in the muscle fibres or supporting tissue, no hæmorrhages, or evidences of degeneration or necrosis of cells, could be found. Yet if the mere presence of the bodies of parasites in a tissue is capable of exciting any such inflammatory reaction or causing cellular degenerations, this was an unusually favorable situation to demonstrate these effects. No such changes existing, their absence appears to furnish some evidence that the bodies of malarial parasites do not exert any marked local toxic influence, but that their local action is largely mechanical. Granting that a considerable disturbance of the heart results from the general toxic condition, it seems probable that the extreme cardiac failure observed in the present case resulted from the mechanical obstruction of the circulation by the masses of parasites in the capillary vessels, but the evidence that such a relation existed can hardly be claimed as demonstrative.

In the kidneys, on the other hand, where the effects of a toxic agent were very marked, the number of parasites was comparatively small, hardly exceeding that found in the peripheral blood. In these organs there were distinct signs of severe acute degeneration, referable, not to the presence of parasites, but to the general toxæmia.

The phagocytic process was extremely active in the liver, spleen, and marrow, where the great majority of parasites were englobed in phagocytic cells. The leucocytes were everywhere actively engaged in the process. In the kidney the majority of phagocytic cells were leucocytes. In the heart muscle, and in the connective tissues generally, the parasites were very abundant and were multiplying rapidly, unrestrained by any phagocytic tendency in the endothelial cells.

Case III.—*Æstivo-Autumnal Malaria. Marked Cerebral Symptoms and Concentration of Parasites in Capillaries of Central Nervous System. Infection with a single well defined group of Parasites. Developmental stages fully apparent throughout a 48 hr. cycle. Severe degenerative changes in the cells of the Convoluted Tubules, with absence of parasites in the renal vessels. Capillary Varicosities in the Liver, with atrophy of parenchyma.*

B. T., 32 years. While in Cuba he had attacks of chills, fever, and diarrhœa, but partly recovered under quinine. On the transport the same symptoms returned. After arrival at Camp Wikoff he suffered from nearly continuous fever without chills, and the diarrhœa became more profuse.

On admission to the General Hospital, September 6, he was considerably emaciated, slightly jaundiced, and completely prostrated. He was given 2½ gr. of bimuriate of urea and quinine, subcutaneously, t. i. d., and

opium. He passed a restless night and the temperature remained high. At 10 A. M., September 7, examination of the blood showed a recent sporulation of parasites. The temperature fell steadily during the day but the patient became delirious in the afternoon, and comatose by night, and never recovered consciousness. There was a moderate rise of temperature on Sept. 8, while the coma deepened and the pulse gradually failed. Death occurred at 3 A. M., Sept. 9.

Blood Examination, September 7, 10 A. M., showed a great many æstivo-autumnal ring-shaped parasites, 2-3 μ in diameter, lying in shrunken red cells. Most of them are entirely free from pigment, but rarely one or more fine pigment grains may be detected. A great many of the rings are less than 2 μ in diameter. Multiple infection of cells is not unusually frequent. In the fresh condition the parasites exhibit rapid changes in outline but few distinct pseudopodia.

The red cells show the changes of a severe secondary anæmia with beginning changes in the size of the cells. The leucocytes are reduced in number. No eosins seen. No pigmented leucocytes.

Sept. 8, 10 A. M., the parasites are much less numerous. They are nearly all of larger size, 4-5 μ, and maintain the form of a ring with thickened irregular segments. A few show one or two fine pigment grains. The chromatin is variously subdivided and usually displaced from the periphery of the ring, being often found as a small group of very fine granules in the centre of the ring, or arranged in the form of a crescent or figure 8, or as an irregular mass or group of granules lying at some distance from the ring. No spheroidal bodies with compact pigment mass and no rosettes were seen in this case. Several hours were spent at various times in the study of these specimens and during that time two crescents were encountered. The unity of the group of parasites is to that extent imperfect.

In the fresh condition the formation of pseudopodia and the amœboid motion are very active, and the pigment grains show slight vibratory motion. The leucocytes are as before.

Autopsy.—4 hours after death. *Body* markedly emaciated, slightly jaundiced; no œdema. *Lungs* show old emphysema and hypostatic congestion. *Heart*, rather small; valves and muscle normal; pericardium distended with clear serum. *Spleen*, slightly enlarged, moderately pigmented, dark brown, rather soft. *Kidneys*, about normal in size, consistence reduced, capsules not adherent, surface smooth, cortex somewhat thickened, pale, markings regular but indistinct. *Stomach*, contains bile, otherwise negative. *Intestine*, normal. *Brain*, no increase of serum, no venous congestion, no dropsy of ventricles; cortex slightly brownish on section; no petechiæ.

Microscopic Examination.—Liver.—The liver cells are very fatty, and contain many coarse yellowish granules, some of which give the reaction of hæmosiderin. No necrotic foci were seen. In many lobules the cords of liver cells are partly or completely atrophic and the capillaries are much widened, forming a variety of cavernous tissue. These changes are of irregular distribution in the organ, being sometimes most marked about central veins, but more often affecting large irregular portions of lobules. Pigmentation of endothelial cells and of leucocytes is marked but not extreme. Parasites are very scarce, but a few small spheroidal bodies and minute rings could with difficulty be identified.

Spleen.—Appearances similar to those of Case II; but very few parasites could be identified.

Marrow.—The marrow of the vertebræ is very fatty. In the cellular cords, there is in places moderate proliferation, and the cells appear in compact masses. Generally, however, the cords appear normal or deficient in colorless cells. The pigment deposit is slight and no parasites could be identified. In smears of the marrow a few ring-shaped parasites within red cells were identified. The nucleated reds, eosins, and giant-cells are very deficient.

Kidneys.—The convoluted tubules are markedly dilated and filled with granular coagulum. The cells are flattened, or broken and degenerated, and nearly all contain great numbers of large and small light yellowish granules which give the Prussian blue reaction of hæmosiderin. The capsules of the glomeruli are considerably dilated. The capillary tufts contain a moderate number of pigmented cells and a few spheroidal parasites. In a few of the ascending limbs of Henle's loops there are a good many clumps of pigment lying within the lining cells, but this condition is not at all frequent.

Brain.—Throughout the cerebrum, cerebellum, medulla, and upper cervical cord, the capillaries contain a very large number of red blood cells harboring parasites. Most of these are small pigmented spheroidal bodies; some exhibit the large ring form with little pigment, and a very few rosettes were identified. The pigment deposit, outside of the parasites, is slight. A considerable number of capillaries were found completely filled and apparently occluded by masses of infected red cells, pigmented leucocytes, and swollen pigmented endothelial cells. In the same regions the small arterioles and all the larger vessels were almost entirely free from parasites. The ganglion cells everywhere show reduction in size, irregularity, splitting, or loss, of chromatic bodies. These changes are less marked in the large stichochromes of the bulbar nuclei than in the cerebrum and cerebellum.

Epicritical:—Although further evidence is hardly required to demonstrate the fact that in many cases of pernicious malaria of the cerebral type the capillaries of the brain contain an excessive number of parasites, the present case is such a striking example of this condition as to be worthy of record.

The relation of the cerebral symptoms can apparently be closely connected with the development of the parasites as followed in the examinations of the blood. Sporulation occurred during the night of September 6, when the temperature was at its highest point, 104°. At 10 A. M., September 7, when the patient was extremely restless, the blood and presumably the brain contained a large number of small ring-shaped parasites. Delirium and partial stupor began on the same afternoon. At 10 A. M., September 8, when the patient was comatose, the parasites had markedly increased in size and many had retired from the general circulation. At this time it is reasonable to infer that the

increased size of the parasites and probably their increased numbers in the central nervous system had seriously impaired the capillary circulation. At death, 3 A. M., September 9, preceded for several hours by profound coma, the sections of the brain show that the majority of the parasites had reached their full development, some were segmenting, and many cerebral capillaries were occluded.

The presence of a single group of parasites, the development of which could be followed throughout the cycle, is one of the interesting features of the case. Sporulation appears to have been completed during the night of September 6, when the temperature reached its highest point, 104°. At 10 A. M., September 7, the blood contained a large number of rings nearly all under 3μ in diameter, with a single large chromatin body, and without pigment. These parasites appeared to have had at least 6-10 hours' growth. At 10 A. M., September 8, the parasites had increased in size, measuring about 4-5μ in diameter; numerous outgrowths had appeared on the circumference of the rings; the chromatin (Nocht's method) was invariably increased in quantity, subdivided, and irregularly placed, and a few parasites showed slight pigmentation. There were still no spheroidal bodies, with compact pigment, to be found, after 30 hours' growth. The patient died at 3 A. M., September 9, and the great majority of parasites found in the cerebral capillaries were of large size and abundantly pigmented, and a few rosettes were seen, indicating the approach of general segmentation at the end of 48-50 hours' growth. Judging from the appearance of the parasites found in the sections of the brain it would appear that about 6-10 hours' growth separated considerable numbers of the youngest from the oldest members of the group, although between a few individuals the intervals must have been much longer.

The severity of the renal lesion, with the absence of parasites in the renal vessels, also requires mention. The changes in the cells of the renal tubules were more advanced than in any other uncomplicated case of the series and appeared to be purely of the type of acute degeneration. The lining cells were markedly eroded and largely composed of a multitude of light yellow granules giving the reaction of hæmosiderin. This destruction of the lining cells caused the dilated tubules to be more or less filled with granular detritus, but there was no further evidence of an exudative process. The kidney was free from chronic changes. In the absence of parasites and of signs or causes of acute inflammation, this lesion must be referred to a toxic condition associated with the malarial infection.

The evidence of the present case, therefore, fully accords with the conclusion drawn from other cases of the series, that the usual renal lesions of pernicious malaria are referable to the effects of a toxic process and not to the direct action of parasites.

Case IV.—*Æstivo-Autumnal Malaria. Extreme Malarial Infection. Localization of Parasites in Bone Marrow. Infection with only one group of Parasites. Pernicious Anæmia.*

F. J., 27 years, private, U. S. Army. No important details of the history of this case were obtainable. It was learned that the patient had suffered severely from malarial fever in Cuba and on the transport, and was received at the hospital at Montauk in a precarious condition, dying a few hours afterward in spite of stimulation and subcutaneous injections of quinine.

Autopsy.—6 hours after death. *Body* very much emaciated, markedly jaundiced. *Lungs*, lower lobes œdematous; commencing hepatization of lower part of right upper lobe. *Heart*, flabby, pale; valves normal; pericardium distended with clear serum. *Liver*, of moderate size, almost black; gall-bladder distended with bile. *Spleen*, moderately enlarged, soft, black. *Kidneys*, slightly enlarged, surface smooth, capsule not adherent, cortex thick, yellowish, markings regular, indistinct. *Gastro-intestinal tract*, negative. *Peritoneum*, slate colored. *Marrow* of ribs and vertebræ chocolate colored.

Microscopic Examination.—Blood.—The only specimens secured were squeezed from the incised finger-tip at the autopsy, six hours after death. The red cells are extremely deficient in number and usually fail to form rouleaux. The plasma stains slightly. There are extreme differences in the size of the red cells, which vary from small microcytes to very large megalocytes; most are larger than normal. Nearly all cells contain an increased quantity of Hb. and many are polychromatophilic. Nucleated red cells are abundant and nearly all fall in the class of megaloblasts or gigantoblasts. There is a pronounced polynuclear leucocytosis (antemortem?), but eosinophile cells are scarce. Many leucocytes, both mononuclear and polynuclear, are pigmented. Comparatively few parasites could be found, and these were all small rings, as seen in the smears of the marrow.

Liver.—There is slight fatty degeneration of the liver cells; the chromatic network is irregular and indistinct, and the cells all contain large greenish granules which give the reactions of bile pigment. There are no areas of necrosis. The deposits of malarial pigment are abundant and very similar to those described in Case II, but the evidences of inclosure and destruction of parasites are very much less marked. Very few parasites could be identified.

Spleen shows the usual changes of acute malarial infection.

Marrow.—That of the ribs and vertebral bodies is very cellular. The hyperplasia affects principally the myelocytes, many of which show mitotic figures, other small mononuclear cells, and giant-cells, of which there are many groups of five, six, or more. No fat cells were seen. The sinuses

are gorged with red cells and pigmented leucocytes. Nucleated red cells are very abundant but no "islands" of these cells could be found. The pulp cords contain a great many heavily pigmented endothelial and large mononuclear cells. A few parasites were identified in the sections with difficulty.

In smears of the marrow, all stages of the inclosure and destruction of parasites could be followed in the large mononuclear and endothelial cells. The number of parasites found in these smears is enormous, and nearly all are the small ring-shaped forms without pigment. They are much more numerous in the marrow than in the blood smears or in any other tissues of the case, and much more numerous than in the smears made from the marrow of any other case of the series. Examples of multiple infection of the same red cell are very numerous, three and four rings being very frequently seen; five and six were sometimes encountered, and one cell was found containing seven distinct young rings.[9] In spite of the enormous number of parasites and the abundance of nucleated red cells, none of the latter were found infected. No crescents were seen. Of the nucleated red cells many are of normal size and appearance, but a great many darkly staining megaloblasts and gigantoblasts are present. The eosinophile cells are about normal in numbers, while the giant-cells are distinctly increased.

The *kidneys* show the lesions of acute degeneration similar to those of Case II, but the number of parasites in the capillaries is small, and no rosettes were seen. Pigmentation is moderate.

In the *heart-muscle* a few ring-shaped and a very few small spheroidal parasites were found.

The *gastro-intestinal tract* exhibited no lesions of importance; its wall contained a moderate number of small parasites without pigment.

The brain was not examined.

Epicritical:—In this case there are several isolated features of interest. The localization of an enormous number of parasites in the marrow of the ribs and vertebræ is a peculiarity not encountered in any other case of the series. It has appeared from smears and sections of the marrow of other cases that the activity of phagocytes in this tissue, as in the liver and the spleen, greatly reduces the numbers of parasites that can be demonstrated in smears of these tissues. This general rule, first formulated by Guarnieri and Bignami, has applied in all other cases of my series, but in the present instance no large number of parasites was demonstrated in any other tissue except the marrow, where the excessive pigmentation is further evidence of unusual activity of the parasites in this locality.

Attention may here be drawn to the necessity of relying only upon smears of the tissues and not upon the examination of sections to

[9] Shown in Plate XXX, Fig. 2, in *Journal of Experimental Medicine*, 1901, v.

determine the number of parasites present in tissues which are actively engaged in phagocytosis. Although formalin as used in fixing the tissues proved to be a very superior agent for this purpose, it has always seemed to me difficult and hazardous to attempt to identify parasites in stained sections of the liver, spleen, or marrow, and in many instances where none could be identified in sections, smears of the tissues revealed their presence in abundance. In the kidney, heart-muscle, brain, and all tissues where phagocytosis is less active, the results of examination of stained tissues appear to be fully reliable, especially if Nocht's method is used.

A further point of interest in the present case is the evidence pointing to the existence of a single very compact group of parasites in the blood and viscera. The forms found in the blood and in the smears of the viscera included only the smaller non-pigmented ring-shaped parasites. Neither pigmented spheroidal bodies, nor rosettes, nor crescents, were anywhere seen. This condition is the more remarkable since the pigmentation of the viscera, the grade of anæmia, and the general condition of the patient indicated a somewhat prolonged course of the infection.

The changes in the blood in this case are of special interest, illustrating the rapid development, as a result of malarial infection, of a condition identical in morphological character with that of primary pernicious anæmia. As the first cases of malaria among the troops at Santiago developed in the second week of July, this patient, dying on September 10, could not have been ill longer than eight, or possibly nine, weeks. Yet in this period a condition of the blood was established indistinguishable from that of primary pernicious anæmia, including the abundance of large megalocytes with excess of Hb. and the presence of many megaloblasts. Even the smaller red cells exhibited an apparent excess of Hb. Apart from the lesions directly referable to the growth of malarial parasites, the changes in the marrow included a well-marked cellular hyperplasia affecting principally the myelocytes and other small mononuclear cells, and giant cells; disappearance of fat cells; increase in size of the nucleated red cells, the majority of which were megaloblasts; and disappearance of "islands" of nucleated red cells.

Case V.—*Æstivo-Autumnal Malaria. Massing of Parasites in Kidneys. Acute Hæmorrhagic Nephritis. Casts Entangling Infected Red Cells and Pigmented Leucocytes in Discharging Tubules. Infection with a single very compact Brood of Parasites.*

M. S., female, aged 17, contracted malaria on Long Island, and after a short period of mild paroxysms was completely prostrated on Sept. 12, 1900. On admission to hospital, Sept. 15, the chief symptoms were prostration, restlessness, vomiting, and moderate œdema of legs. Temperature 104.2° F. Urine, of high color, sp. g. 1018, contained many epithelial cells, detritus, and many red blood cells. Diazo-reaction marked. Widal's reaction absent. Bowels costive. Restlessness and vomiting were partly controlled by sedatives. The vomiting of a round worm led to the administration of three full doses of santonin on Sept. 19. Mild delirium developed into coma on Sept. 20, and with failing pulse and diminished, bloody urine the patient died on Sept. 20. There were two complete remissions of fever on Sept. 18 and 20, but no chill occurred. *Clinical diagnosis*, typhoid fever and acute hæmorrhagic nephritis.

Autopsy.—By Dr. Otto Schultze, 14 hours after death. Body well nourished, rigor firm, slight œdema of legs. *Heart*, *lungs*, *stomach*, and *pancreas* normal. There was one ounce of straw-colored serum in the pericardium, and a few ecchymoses in the visceral pleura and pericardium. *Intestine*, ileum congested, lymph-follicles slightly enlarged; colon normal. *Liver*, without gross indications of malarial infection, was pronounced normal. *Brain* normal. *Spleen*, 16 oz., firm, dark red, with prominent Malpighian bodies. *Kidneys* weighed together 16 oz.; cortex much thickened, capsule free, markings obscured, color very light; medulla intensely congested, in places rusty; in cortex of right kidney a superficial anæmic infarct with surrounding hæmorrhagic zone, measuring 3 x 2½ cm. in area, and 3 mm. in depth.

No gross evidences of malaria were detected.

Anatomical diagnosis.—Acute hæmorrhagic nephritis.

Cultures from liver and spleen yielded Bacillus coli communis, but no growth of Bacillus typhosus was obtained.

Through a misunderstanding all the viscera except the kidneys were thrown away. The kidneys were hardened in 5 per cent formalin.

Microscopic Examination (Plate XIII).—The lining cells of the convoluted tubules exhibited a very advanced stage of degeneration of a peculiar type. The cells were greatly swollen, their outlines obliterated, so that the distended tubules were filled with a coarsely reticulated mass of cell-detritus in which pyknotic nuclei were irregularly scattered. The degenerative process was principally hydropic, little fat or stainable protoplasm being present (Plate XIII, Fig. 13). There were many minute foci of necrosis, especially in the right kidney. The infarcted area was in an early stage of coagulative necrosis, and throughout this area, especially in the hæmorrhagic zone, the vessels were distended with blood containing enormous numbers of small pigmented parasites. Many small vessels leading to the infarct were distended to two or three times their normal calibre by thrombi of infected red cells. The capsules of the glomeruli were distended with granular coagulum (Plate XIII, Fig. 13). Most of the cortical capillaries were collapsed by the distended tubules and free from blood and parasites. Throughout the medulla were many miliary hæmorrhages, and most of the capillaries were distended with red cells, most of which contained parasites, or with thrombi of pigmented parasites which

had nearly destroyed the red cells. The parasites were remarkably uniform in size, being full grown æstivo-autumnal forms with abundant pigment. A very few rings and rosettes were seen. The discharging and many higher tubules were distended with granular, epithelial, or blood casts. Some of these casts had entangled leucocytes, often pigmented, and infected red cells, while some infected red cells lay free in the lowest discharging tubules. From their low position in the tubules all of these casts must have reached the urine in at least moderate numbers. The remarkable massing of parasites in the renal capillaries is shown in the photographs (Plate XIII, Figs. 14 and 15).

Epicritical:—The absence of microscopic examination of the other viscera, while greatly to be deplored for the sake of completeness in the report of this case, cannot seriously detract from the importance of the findings in the kidneys. Doubtless there were many parasites in the other viscera, but in the absence of gross evidence of their presence it is safe to conclude that these viscera were not the seat of any special localization of parasites. This conclusion is rendered more trustworthy from the fact that the single compact brood in the kidney was composed of full-grown forms which were richly pigmented.

Moreover, numerous miliary hæmorrhages, and thrombosis of vessels with infarction, are the usual mechanical results of the presence of enormous numbers of parasites in a tissue, and the great extent of these lesions in the kidneys throws this case in the same class with the comatose and choleraic cases, with localization of parasites in the brain and gastro-intestinal mucosa respectively. The extreme degeneration of the tubule cells seems in part referable to the general toxæmia of the disease, but its extreme degree and peculiar character were not seen in any other case of the series, and may, with considerable certainty, be attributed to the obstruction of the circulation by the thrombi of infected red cells. Since the discharging tubules contained, among a large number of casts, some entangling infected red cells and pigmented leucocytes, while a few infected red cells were found free in the lumina of the tubules, it seems possible that a diagnosis of such a case might be established during life from the examination of the urine, which should show marked diminution in quantity, considerable albumin and blood, many granular, epithelial, some blood casts, and *infected red cells* and pigmented leucocytes, both free and adherent to casts.

Case VI.—*Æstivo-Autumnal Malaria (?). No Parasites found in Blood. Few Parasites and unusually little evidence of malarial infection found in the Viscera.*

B., age 32. Served with regiment in Santiago campaign and contracted malarial fever in Cuba. Stated that he had four attacks in Cuba, marked

by chills and fever. The last and longest illness lasted three weeks. Was treated always by quinine with good results, but on omitting treatment the disease promptly relapsed. Recently he had suffered from mild chills daily, with more continued fever. On Sept. 4, the day of admission, he had a slight chill at 8 A. M. and that afternoon the temperature was 101.6°; pulse, 80.

Physical examination was negative, the spleen not being found notably enlarged. The patient was not markedly emaciated or anæmic. All symptoms pointing distinctly to malaria the patient was given quinine, but next morning, September 5, he had another chill and the temperature rose to 104°. Quinine was then increased, and given subcutaneously in large doses. On September 8, there was a moderate chill and rise of temperature. The stools were now diarrhœal, the abdomen moderately distended, the skin slightly jaundiced, and as the fever did not yield to quinine the blood was examined.

Blood Examination. Sept. 8.—The red cells showed the changes of well marked secondary chlorotic anæmia. The leucocytes were reduced in numbers. No eosinophiles. No distinct pigmented leucocytes, but a few contained single grains of pigment apparently malarial. In spite of prolonged search no parasites could be found. This result was confirmed by several subsequent examinations of the same and other specimens. The dissolved blood in the proportion of about 1 to 10 of water was mixed with a broth culture of B. typhosus, yielding prompt and typical clumping.

On the evidence of a temperature resisting quinine, of typhoidal stools and abdominal symptoms, absence of parasites from the blood, and presence of Widal's reaction, the administration of quinine was stopped from September 9 to 10 inclusive. The temperature declined, but the other symptoms persisted and the patient grew steadily weaker.

Blood examination, Sept. 9, gave the same result as before, but the Widal test was not repeated. Sept. 10, temperature fell to 99.6°, but the patient's condition was not improved. Quinine was again administered subcutaneously. Sept. 11, abdomen more distended; diarrhœa continues; no eruption; patient at times mildly delirious; pulse very weak. Blood examination, Sept. 11, showed no parasites, but a few characteristic pigmented leucocytes were seen. Sept. 12, patient died. The diagnosis was at this time regarded as probably typhoid fever.

Autopsy.—10 hours after death. Body moderately emaciated, slightly jaundiced. *Lungs*, moderately congested and œdematous. *Heart*, left ventricle slightly dilated, wall rather thin, flaccid; old thickening of mitral and aortic valves. *Liver*, moderately enlarged, moderately dark brownish in color, but not slaty. *Spleen*, moderately enlarged, firm, dark red in color. *Kidneys*, enlarged, capsule free, surface smooth, cortex thickened, pale; markings regular but indistinct. *Gastro-intestinal tract*, shows no lesions of importance; Peyer's patches are not enlarged or congested; mesenteric lymph nodes are not enlarged. The stomach contains blackish fluid but there are no evidences of gastro-duodenitis. *Brain*, in the absence of cerebral symptoms, was not examined. *Marrow*, hyperæmic, but shows no gross indications of malarial infection. *Blood*, moderately anæmic, but not otherwise changed in color.

Microscopic Examination.—Spleen shows the usual changes of acute malarial infection. The deposit of pigment, although considerable, is much less than usual in fatal cases of malaria. Smears of the splenic pulp made at the autopsy showed the presence of a very few ring-shaped parasites in red cells. The pigmented leucocytes and endothelial cells are of the usual character in acute malarial infection.

Liver.—The deposit of pigment is rather less abundant than in the spleen and is limited to the leucocytes and endothelial cells. The liver cells show destruction of chromatic network, and a moderate deposit of greenish granules; some contain fat globules. In the smears from the liver, no parasites could be identified, but some atypical intracellular pigmented spheroidal bodies were noted which may have been malarial parasites.

Marrow.—The deposit of pigment is scanty. Fat cells are moderately abundant in the ribs, but the evidences of cellular hyperplasia are distinct. In the smears some typical macrophages englobing parasites in all stages of digestion, are found. A few infected red cells are seen. The nucleated red cells, eosins, and giant cells, are deficient in number.

In the other viscera the deposit of pigment was much less abundant than in most cases of fatal malaria, and no parasites could be found.

Epicritical:—While the data in this case must be regarded as inconclusive in important respects, the facts demonstrated render the case one of unusual interest and obscurity.

The most important feature is the absence of parasites from the blood, as repeatedly demonstrated, in a patient who nevertheless died with a typical temperature curve of fatal æstivo-autumnal malaria. The absence of parasites in this instance is not fully explained by the previous treatment with quinine, as in all other cases of my series in which all parasites disappeared promptly after quinine, the patient improved. Moreover, it is an almost invariable experience that cases of malaria that die without parasites demonstrable in the blood are more chronic cases complicated by severe anæmia or cachexia. All the other fatal cases of acute malaria seen at Montauk showed many parasites in the blood at death, although many of them had been treated with very large subcutaneous injections of quinine for as long a period as in the present case. Accordingly the absence of parasites from the blood, under the conditions existing, at once raised a doubt regarding the nature of the disease.

The possible existence of typhoid fever was completely set aside by the autopsy, unless one assumes an infection by B. typhosus without intestinal lesions.

Both the gross and the microscopic examinations of the viscera, however, as well as the clinical history, indicate as the cause of death an acute malarial infection in the fourth or fifth relapse, proving fatal

with very few parasites in the blood, which disappeared promptly on the administration of quinine. The present case therefore seems to fall properly in a rare and obscure class described by Marchiafava, Bignami, and Bastianelli[10] (1894), in which few parasites were found in the blood during an acute fatal attack of malaria, and in which no distinct gross signs of malarial infection were found at autopsy, the diagnosis requiring a microscopic examination of the viscera.

These cases occurred in July or late in the summer. They usually presented the rather typical clinical symptoms of pernicious malaria, especially the cerebral symptoms. On examination of the blood, however, even early in the disease, very few parasites (one or two in a single slide) or, later, none at all, were to be found, while the viscera showed no distinct gross lesions of a previous severe malarial infection. On microscopic examination of the viscera, pigment deposits were unusually scanty and few or no parasites were found.

These cases, according to the authors, are not to be classed with those in which the parasites gradually disappear in severe acute infections, none being found in the finger blood at death although the viscera exhibit abundant evidence of malarial infection; nor yet with those in which the parasites disappear after a long infection has spent itself. They believe that the fatal issue is rather to be referred to general debility of the patient, or to an unusually virulent infection, or to an occasional combination with sunstroke.

In my case the patient had not suffered from the campaign more than had many others, and although he had undoubtedly been living under very unfavorable conditions, this fact, though important, seems insufficient of itself to explain the unusual features of the disease. Neither was there any indication that the patient suffered unusually from the heat which prevailed at that time, even at Camp Wikoff. Finally, a virulent malarial infection usually produces very large numbers of parasites, severe anæmia, and heavy deposits of pigment, all of which were absent, at least in the fatal attack.

If the present case is to be accepted as one of pernicious malaria, and it is essentially similar to those of the Italian authors, the conclusions must then be drawn that æstivo-autumnal malaria may pass through four or five relapses without leaving marked deposits of pigment in the viscera and may end in a fatal attack in which very few parasites, which soon disappear under quinine, are to be found in the blood or viscera.

After referring to their own cases of this character Marchiafava

[10] loc. cit.

and Bignami take pains to state that after years of experience they believe that fatal malaria does not exist without parasites in the finger blood. The present case cannot be regarded as an exception to this rigid rule, and I would particularly point out the fact that the patient's blood was not examined until 12 hours after the beginning of treatment by subcutaneous injections of quinine.

Case VII.—*Fatal Malaria from Infection with Large Tertian Parasite (Golgi). Extreme Anæmia. Prolonged Coma without Parasites in Brain. Catarrhal Colitis. Hæmoglobinuric Malarial Fever.*

G. F., aged 32, private, U. S. Army, contracted malarial fever at Santiago in July, 1898, and had had repeated relapses with short intervals of improvement, when he had felt able to return to duty. Had often experienced severe shaking chills at somewhat irregular intervals. Had steadily lost flesh and become very anæmic. About Sept. 11, was again prostrated with severe chills and fever which failed to respond to quinine administered by the mouth or subcutaneously. On admission to General Hospital at Camp Wikoff, Sept. 13, the patient was comatose, and remained so until death at 3 A. M., Sept. 16. When seen by me, Sept. 15, the patient was comatose and moribund. The foregoing items of history were obtained from Dr. S. W. Allen, of Boston, who had seen the patient on admission, and to whom I am indebted for the opportunity of studying the case.

Autopsy.—8 hours after death. Body much emaciated, excessively anæmic, and slightly jaundiced. Chocolate-colored fluid exudes from mouth. *Lungs*, anterior portions anæmic, posterior much congested, moderately œdematous, and unusually dark in color. *Heart* muscle very flabby, of pale brownish tinge; valves normal. *Blood*, very watery, of slightly brownish tinge, coagulating feebly. *Liver*, not distinctly enlarged; of slaty color but not extremely dark; outlines of lobules very indistinct; gall-bladder distended with bile. *Spleen*, greatly enlarged, soft, but not diffluent; of typical chocolate brown color. *Pancreas*, small, dark salmon in color, rather soft. *Kidneys*, slightly enlarged, surface smooth, cortex thick, deep red, in places rusty, markings indistinct; organ very greatly congested. *Stomach*, partly digested in places, contains some dark bloody fluid. *Small intestine*, exhibits no abnormalities. *Colon*, lower third the seat of an intense catarrhal inflammation with superficial erosions, and swelling of solitary follicles. *Mesenteric lymph-nodes* are not enlarged. *Serous membranes*, slightly darkened and jaundiced, and contain slight serous effusions. *Marrow*, very hyperæmic, of chocolate brown color. *Brain*, white matter perhaps slightly darker than usual; dura and pia yellowish; no effusions, no petechiæ. Specimens preserved in 5% formalin.

Microscopic Examination.—Blood. Sept. 15. Smears contain a very large number of tertian parasites in all stages of development. These parasites are of large size, richly pigmented, except in the very young forms; their nuclei fail to stain by methylene blue, and the infected cells are much swollen and distinctly pale. A prolonged search, repeated on several subsequent occasions, failed to show the presence of any bodies that could be classed with the æstivo-autumnal parasite. All the ring forms were coarse,

usually pigmented, with a large achromatic spot (methylene blue), and the infected cells were swollen.

The red cells show the changes of a severe secondary chlorotic anæmia. There was no leucocytosis, but the eosinophile cells are not relatively numerous. A moderate number of pigmented leucocytes were seen.

At the autopsy, smears upon glass slides were made from the expressed marrow of the ribs, and from the splenic pulp.

Smears from the marrow contained an abundance of pigmented cells. The pigment is usually darker and finer than that seen in æstivo-autumnal infections. When occurring in clumps, the clumps are rather more compact, but most of the phagocytic cells contain more diffusely scattered pigment grains than are seen in æstivo-autumnal cases. Many rods and clumps, however, are indistinguishable from the æstivo-autumnal pigment. Very few infected red cells could be found, but there were a few small pigmented intracellular bodies staining faintly with methylene blue. Nucleated red cells, eosinophile cells, and giant-cells are distinctly deficient.

Smears from the spleen contain a great number of pigmented cells similar to those of the marrow. There are a large number of distinctly stained pigmented parasites of considerable size. Very few or none of the younger parasites seen in the blood could be identified in these smears. No vacuolated parasites could be found, but there was a considerable variety of small, vacuolated leucocytes, many of which were pigmented (Plate XI, Fig. 8).

Spleen.—There is extreme pigmentation of all the larger cells, the pigment showing the same characteristics as in the marrow and liver. No parasites could be identified. There is a well-marked cellular hyperplasia of the pulp tissue; the large and small sinuses are choked with pigmentiferous and other cells; the Malpighian bodies are reduced in size, and the blood content of the organ is increased.

Abdominal lymph nodes show moderate hyperplasia, slight pigmentation of endothelial cells, many mast-cells, but no parasites.

Marrow (rib).—The pigment deposit is very heavy, but few parasites could be identified. The cellular elements are very abundant and in places there seems to be some proliferation. There are marked congestion of all vessels and a few small hæmorrhages.

Liver.—There is extreme pigmentation of endothelial cells, macrophages, and leucocytes, but the parenchyma cells are almost entirely free from brownish pigment. The deposit of pigment in the former cells differs from that seen in æstivo-autumnal cases, in being finer and more diffuse (Plate X, Fig. 3). Although there are many cellular inclusions in the phagocytes, no parasites could be positively identified. The liver cells are not fatty, but the chromatic reticulum is faint and irregular, and many cells contain coarse greenish granules. A few small foci of necrosis were found, in the neighborhood of which dilated capillaries filled with large phagocytic cells were sometimes noted.

Kidney.—The blood content of the organ is enormously increased. All the large vessels are gorged with blood, the intertubular capillaries in numerous small areas are greatly dilated and distended with blood. The capillary walls are usually intact, but in many places there are small hæmorrhages into the tubules. Everywhere, especially in the medium-

sized and larger vessels, there are peculiar collections of pigment to be described later. The quantity of ordinary malarial pigment is not large.

The cells of the convoluted tubules show advanced stages of degeneration. In the less congested areas the cells are moderately eroded, and contain a large deposit of fine yellowish granules of hæmosiderin. Near the hæmorrhages and between markedly dilated capillaries, the tubule cells are often necrotic, the nuclei fail to stain, and the protoplasm is broken up into shiny opaque globules staining with eosin. Sometimes the entire cellular lining is fused into a homogeneous opaque mass entirely detached from the membrana propria. In some lining cells and in some tubules there are a few pigmented parasites, which are also to be seen in the capillaries.

Lungs.—All capillaries contain an enormous quantity of granular and crystalline pigment in leucocytes, endothelial and epithelial cells, and free in the vessels. No parasites were identified.

Stomach and *small intestine*, negative. The inflamed *colon* shows intense congestion of mucosa, exfoliation of epithelial lining in places, moderate pigmentation of leucocytes and of endothelial cells. No malarial parasites identified and no Amœbæ dysenteriæ found.

Heart muscle.—In the capillaries of the heart muscle there are a good many richly pigmented leucocytes, considerable deposits of crystalline pigment, but very few parasites could be found, either in the red cells or leucocytes, or in the plasma. There is considerable artificial separation of muscle cells in some places, but the tissue shows no evidence of inflammation nor the cells any marked signs of degeneration. Many of them show a deposit of greenish granules about the nucleus. There are a great many huge mast-cells in the endomysium.

Brain.—Throughout the cortex, medulla, and cerebellum, there is the usual hyperæmia, but the great majority of all vessels are entirely free from pigment and parasites. In a good many capillaries an isolated, pigmented, intracellular spheroidal body is found, of slightly larger size than those seen in æstivo-autumnal cases. The pigment in these bodies usually appears in coarse dark grains. In a very few capillaries, there are masses composed of a few pigmented spheroidal parasites, pigmented leucocytes and swollen pigmented endothelial cells, capable of partly obstructing the circulation in the capillary, but these obstructed vessels are so scarce that it seems hardly possible to refer the cerebral symptoms to this condition.

There is a uniform reduction in the size and number of chromatic bodies in the ganglion cells and considerable post-mortem clouding.

Epicritical:—While the statement of Italian authorities has long held true that no autopsy has been reported in a case of malaria with infection by the large tertian parasite, as the infection is never fatal, the present case requires a modification of this view, offering an instance of fatal uncomplicated malaria with infection by the large, and so-called "benign" tertian parasite, demonstrated in the blood during life in large numbers and without admixture with other varieties, and indicated further by the somewhat peculiar character of the pigment deposits in the viscera

The above claim of the Italian observers, although based on a very wide knowledge and experience, must be regarded as too sweeping, since almost any condition may occasionally prove fatal through accidental complications. Thus, Barker[11] has reported a case of tertian malaria with autopsy, in which death was referable to general infection with Streptococcus pyogenes.

In another case seen by me at Montauk, death occurred in the third relapse when the blood contained a great many large tertian parasites, and no other organisms were found after repeated and prolonged examinations. Death in this case was undoubtedly due to the growth of this usually benign parasite, but in a subsequent review of one of the specimens, a single crescent was encountered, necessarily throwing the case in the class of double infections, according to the present theory of malarial species, but not seriously vitiating its value as a proof that the large tertian parasite is occasionally malignant.

The severity of the anæmia in the present case also deserves mention, in view of the general rule that the large tertian parasite is less active in impoverishing the blood than the malignant tertian. Although the Hb. was not estimated nor the cells counted, the pallor of the patient, the anæmia of the viscera, and the watery condition of the blood in the large vessels indicated a state of anæmia which was not exceeded in any other case of the series.

The prolonged coma, with very few parasites and very little pigment in the brain, classes this case with others which indicate that profound cerebral disturbance in acute malaria is not necessarily connected with the localization of parasites or pigment in the cerebral capillaries.

The catarrhal colitis and its cause are a feature of interest in this case. The microscopic examination failed to reveal any evidence that the malarial infection was directly concerned in this lesion, as few parasites and little pigment were found in the wall of the colon.

The character of the pigment in the case deserves special attention. As already mentioned, its occurrence in finer and darker grains more diffusely scattered throughout most phagocytes, especially in the hepatic endothelium, appears as a feature which may serve to distinguish the deposits of tertian from those of most æstivo-autumnal infections. Throughout the viscera, in the small and medium-sized vessels and in some capillaries there were deposits of a peculiar form of pigment somewhat similar in color to, but different in form from, that elabor-

[11] Op. cit.

ated by the malarial parasite. Sections of some of the injected vessels appeared almost black from deposits of this material. On examination this pigment appeared in the form of fine or coarse grains, or short rods, or short acicular crystals of dark brown color. It was usually attached to the surface of the red cells, but much was free in the plasma. Various stages of its formation could be followed in the blood vessels of the kidney, pia mater, and heart-muscle. In the earliest stage noted the red cells were found to be fringed with a row of small dark granules, while the body of the cell exhibited a faint yellowish-brown tinge. This appearance was often limited to the central portions of the mass of blood in the vessels, while adherent to the walls were leucocytes containing malarial pigment of the ordinary character (Plate XII, Fig. 11).

When more abundant, the pigment grains were usually of larger size and in quantity sufficient to fill the interspaces between the red cells. In this case the section of the injected vessel sometimes appeared opaque and brownish. Usually the deposit was very abundant and the red cells were covered with rod-shaped granules or short acicular crystals lying in or on the cell, or in the plasma (Plate XII, Fig. 12). Such cells sometimes exhibited a diffuse dark-brown color. Radiating groups of finer acicular crystals were commonly found in the richer deposits. In the kidney and heart muscle many arterioles were almost black with the deposits. No parasites were found in any of the red cells affected by the process. The bodies of the leucocytes were usually free from pigment grains of this character, but many were surrounded by numerous crystals, especially in the capillaries.

The distribution of the deposits was peculiar. They were found most abundantly in the heart muscle and kidney; the earlier stages were noted in the lungs, pia mater, and abdominal fat and lymph nodes: a few crystals were seen in the marrow; while the liver and spleen, containing an unusual quantity of ordinary malarial pigment, failed to show any trace of this crystalline variety.

These extensive deposits of pigment which were found in nearly all parts of the general circulation seem, without doubt, to be referable to a greatly increased globulicidal action of the plasma, and taken in connection with the renal lesions, indicate that the patient was suffering from that extreme form of destruction of blood which characterizes hæmoglobinuric malarial fever.

Case VIII.—*Æstivo-Autumnal Malaria. Amœbic Colitis. Extreme Malarial Infection. Absence of Pigment and Parasites from the Wall of the Colon. Extensive Growth of Amœbæ.*

J., aged 21, private, U. S. Army. When received at the General Hospital, Sept. 9, on his return from Santiago, the patient was too ill to give an account of himself. He was then in a typhoid state, extremely emaciated, with incontinence of fæces, and mild delirium. Temperature 103°, pulse feeble. The temperature fell to 97° in the course of 48 hours, but the patient showed no tendency to improve. He was given moderate doses of quinine by mouth, and the diarrhœa was imperfectly controlled by astringents. The condition was at all times hopeless and he died Sept. 19.

Autopsy.—Two hours after death. Body extremely emaciated and anæmic. *Lungs*, show areas of œdema and hepatization. *Heart*, no gross changes. *Liver*, not enlarged; color very dark brownish, slaty; outlines of lobules indistinct; gall-bladder distended with bile. *Spleen*, very large, rather firm, of very dark chocolate color. *Stomach*, small, contracted. *Small intestine*, negative. *Colon*, lower third the seat of numerous ulcers of moderately large dimensions; bases and edges necrotic and undermined, covered with bloody necrotic material; vicinity in some areas covered with fibrin. Fæces greenish gray in color; semi-solid; contain little mucus; microscopically, they contain a little pus and epithelium, many bacteria, many fat crystals, no pigmented leucocytes, a little food detritus. No amœbæ could be found. *Mesenteric lymph nodes*, much enlarged, hyperæmic, very slightly pigmented. *Marrow*, hyperæmic, of chocolate tinge. *Blood*, watery; shows a chocolate tinge.

Microscopic Examination.—Spleen. Shows the usual lesions of active malaria.

Liver.—There are advanced degenerative changes in the liver cells. The deposit of pigment in various cells is extremely abundant. A moderate number of pigmented spheroidal parasites in the red cells, free in the capillaries, and englobed in macrophages were identified.

Marrow.—In the marrow of the ribs there is an abundant deposit of fresh pigment and several pigmented parasites were identified. Nucleated red cells, eosins, and giant-cells, are deficient.

Blood.—At autopsy found to contain a moderate number of ring-shaped æstivo-autumnal parasites. Red cells showed the changes of severe chlorotic anæmia. There was a moderate polynuclear leucocytosis.

Colon.—The ulcers extend through the mucosa, often to the muscularis, and are floored by a partly necrotic tissue infiltrated with pus and blood. Muscularis is often infiltrated with rows of large round cells. There is very little attempt at healing, and the process seems everywhere to be advancing.

Amœba dysenteriæ is found in considerable numbers in the submucosa. The amœbæ are most abundant beyond the edges of the inflamed areas, groups of them being found where there are no signs of exudative inflammation. In the bases of the ulcers and in the necrosing portions they are absent or very scarce. Evidences of the malarial infection here are very scanty, most of the vessels being entirely free from parasites and pigment. Rarely an isolated slightly pigmented leucocyte is encountered, but no parasites could be found.

There is an extensive growth of bacilli, apparently post-mortem, in and on the ulcerating areas. No cocci could be found in the sections. The complete preservation of the amœbæ, and the absence of post-mortem

changes beyond the walls of the ulcers, as well as the absence of pigment, precludes the possibility that malarial parasites could have been present in the ulcers during life and disappeared very shortly after death (2 hours).

Epicritical:—The present case illustrates a condition rather commonly encountered among the sick at Camp Wikoff on their arrival from Santiago. In the present instance the two protozoan infections appear to have limited themselves strictly to their natural situations and to have had little influence upon each other.

The wall of the colon contained large numbers of amœbæ, while exhibiting almost no indications whatever of the coincident malaria. The liver, spleen, marrow, and peripheral blood, on the other hand, showed the changes referable to an extreme malarial infection quite as severe as that demonstrated in some cases of fatal uncomplicated malaria. If there is any special tendency of an inflamed and ulcerated colon to gather malarial parasites or pigment from the blood, it failed entirely to show itself in this case.

It may be specially noted that while amœbæ were very abundant in the wall of the colon, none whatever were found in the fæces although they were carefully examined under favorable conditions at the autopsy. It should be said that there was very scant mucus, though considerable bloody necrotic material attached to the fæcal matter.

Case IX.—*Tertian Malaria. Chronic Endocarditis. Disposition of pigment three months after subsidence of the active infection.*

J. H., aged 48 years. Had suffered considerably for two years past from dyspnœa on exertion, palpitation, attacks of bronchitis, and transitory œdema of extremities. In June 1897 he had a sharp attack of chills and fever, but recovered under quinine. These attacks were repeated at intervals during the summer but no acute attacks occurred during the winter of 1897-98. In June, 1898, the attacks recurred and the patient was admitted to hospital in a very debilitated condition. The spleen was much enlarged, the anæmia marked, and many tertian parasites were found in the blood. The last acute attack occurred early in July, 1898. Quinine was administered constantly and no further relapses occurred. Meanwhile the symptoms of endocarditis had steadily progressed and on October 11, the patient died.

Autopsy.—One hour after death, by Dr. John H. Larkin, to whom I am indebted for the material from the case.

Heart, much enlarged, chambers dilated, and the mitral and aortic valves markedly stenosed. *Lungs*, very emphysematous. *Liver* showed chronic congestion and fatty degeneration, but no gross indications of malarial infection. *Spleen*, much enlarged, firm, and unusually dark in color. *Kidneys*, the seat of chronic congestion and slight chronic diffuse nephritis.

Microscopic Examination.—Spleen. The Spleen shows moderate evidences of

chronic congestion, in the widening of the pulp sinuses. The Malpighian bodies are distinctly outlined. Most of the pigment is gathered in large conglomerate masses of dark coarse grains lying in and distending the endothelial cells and macrophages about the Malpighian bodies, or in the walls of the splenic arterioles, or in the septa and walls of the veins (Plate XV, Fig. 18). A moderate number of endothelial cells and macrophages in the pulp tissue contain a scanty deposit of yellowish discrete grains. The red cells in both liver and spleen are well preserved, and some contain pigment grains and bluish stained spheroidal bodies which cannot be certainly identified as malarial parasites.

Liver.—There are well-marked lesions of chronic congestion with dilatation of the capillaries in the centres of lobules, with atrophy of the adjoining cells, and fatty degeneration of central and peripheral cells. Most of the pigment is collected in very large intracellular masses either in the portal canals or in the centres of lobules (Plate XV, Fig. 19). A few endothelial cells throughout most lobules contain scattered grains of apparently fresh pigment. The liver cells appear to be entirely free from malarial pigment which is confined to the endothelial cells and large macrophages lying loose in the capillaries, or between the connective-tissue fibres of the portal canals. The pigment grains are usually coarse, dark, and thickly conglomerate, but less often are small, yellowish and discrete.

Marrow.—A few endothelial cells containing coarse black pigment grains were found.

Lungs.—Any malarial pigment present could not be distinguished from the anthracotic pigment of the septa and the fresh blood detritus in the epithelium of the vesicles, belonging to the chronic congestion of the organ.

Epicritical:—The present case is reported to illustrate the condition of the malarial pigment three months after the subsidence of the acute infection. It will be seen that this period had sufficed to remove the traces of malarial infection from most of the viscera, while in the chief depots of pigment, the spleen and liver, the pigment had been largely transferred from the parenchyma to the connective tissue structures, giving sections of these organs a very characteristic appearance (Plate XV, Figs. 18 and 19).

It is to be noted that the above period was not sufficient to enable all the endothelial cells of the liver and spleen to become entirely free from pigment, some of them still presenting the usual appearance of the phagocytes of acute malarial infection. It is possible, however, that these cells were further signs of a partly-suppressed growth of the parasite, uncertain indications of which were also noted in the presence of bluish-staining pigmented bodies found in a few red cells. In appearance the old pigment was considerably blacker than the fresh deposits. It was usually gathered in more compact and somewhat larger spheroidal masses. About the clumps the outlines of an enclos-

ing cell, often enormously distended, could nearly always be discovered, but some of the pigment appeared to be lying free in the lymphatic spaces of the connective tissue.

Most forms of pigment deposited in tissues undergo gradual solution in their inclosing cells, yielding, in the case of iron-holding pigment, a diffuse reaction of hæmosiderin (Ziegler, Thoma, Neelsen). When submitted to the test for hæmosiderin, the cells in the neighborhood of the pigment clumps often gave a more intense blue reaction than was found in the other portions of the liver and spleen. On the outskirts of the clumps the granules appeared very fine, translucent, and of a yellowish tint, but none of these granules gave the blue reaction.

Case X.—*Acute metritis. Purulent peritonitis, from infection with Streptococcus pyogenes. Extreme deposits of pigment in all viscera, closely resembling those of malaria.*

B. D., aged 25. There was no previous history of malarial infection. The patient had always enjoyed good health since childhood. Jan. 2, 1899, the patient was delivered of a healthy infant. She felt well until Jan. 6, when she was suddenly seized with a chill and fever, while the lochial discharge increased in quantity. The physician in charge regarded the condition as referable to malaria and administered quinine by mouth in large doses. The fever persisted and the chills were repeated, but there was no distinct periodicity in their occurrence. A digital exploration of the uterus was made on Jan. 7, and its cavity was found to be free from placental remains. A severe diarrhœa developed but yielded to opium, and was succeeded by constipation on Jan. 12. Jan. 13, another physician was called in, who found signs of general peritonitis, advising removal to hospital. He gave no quinine on Jan. 13 and 14, and it could not be learned how long this treatment had been continued. No local treatment had been used at any time. On admission to Roosevelt Hospital, Jan. 15, the patient was in collapse, with signs of general peritonitis and a moderate discharge from the uterus of chocolate-colored fluid of slight odor. The symptoms were entirely those of peritonitis from puerperal infection. Temp., 104°; pulse, 160; respiration, 38. The urine was acid, sp. g. 1024, contained a trace of albumin, pus, blood, many vesical and renal epithelial cells, but no casts. Death followed a few hours after admission.

Autopsy.—By Dr. Eugene Hodenpyl, 20 hours after death. Body of fairly nourished anæmic and jaundiced subject. No œdema. *Lungs*, much congested, œdematous, with a few small areas of hepatization. *Heart*, muscle pale, flabby, valves normal, chambers slightly dilated. *Liver*, size slightly increased, section smooth, rather darker than usual, outlines of lobules indistinct. Gall-bladder contains 2 oz. of dark bile. *Spleen*, moderately enlarged, very soft, of well-marked slate color. *Kidneys*, slightly enlarged and softened, markings swollen, indistinct; cortex irregularly congested and mottled. *Peritoneal cavity* contains a half-pint of sero-purulent fluid and is everywhere highly inflamed and granular. *Gastro-intestinal tract*, no lesions. *Marrow* of ribs and vertebræ, very hyperæmic, and of dark brown color.

Brain, pia slightly œdematous, vessels gorged with blood, brain tissue jaundiced; no ecchymoses or other abnormalities noted. *Uterus,* slightly involuted, wall rather friable, pale; mucosa deeply congested, of dark red color, covered with a thin chocolate-colored layer of mucus, blood and pus. No remnants of placental tissue to be seen in the cavity of the uterus. Cervix moderately lacerated. No thrombi or pus in vessels of broad ligament.

No cultures were made from the peritoneum or uterus. In smears of the pus from the peritoneum and from the uterine discharge large numbers of cocci in short chains were found. In sections of the wall of the uterus large numbers of cocci in short chains were found, but only on the surface or superficial portions of the mucosa. These cocci were identical in morphology with the ordinary form of Streptococcus pyogenes seen in pus.

Microscopic Examination.—Spleen. There is a very abundant deposit of pigment in the endothelial cells and macrophages of the pulp. In the sinuses there are many richly pigmented leucocytes. The pigment is mostly in the form of grains, short rods, or longer acicular masses. It is very frequently arranged in the form of a small mass of fine brownish grains or crystals. In some places it is more compact and darker. About some of the masses there are minute vacuoles. No malarial parasites could be seen within the red cells, but many contain one or more coarse pigment grains.

Smears of the spleen show abundant pigment, principally in the large mononuclear cells. The pigment is principally granular, but many short crystals are seen. No malarial parasites could be identified.

Liver.—The pigment in the liver is very abundant, occurring in the same form as in the spleen. The liver cells contain considerable pigment in the form of granules, spheroidal or wreath-shaped masses, or elongated rods. Many of the elongated, rod-shaped collections lie in small clear spaces within the liver cells, which contain also much greenish granular bile pigment and show moderate fatty degeneration. No malarial parasites were seen.

Kidney.—There are evidences of acute degeneration of the tubule cells. The convoluted tubules and adjoining capillaries are nearly free from pigment. In the glomeruli are a few pigmented endothelial cells and leucocytes. In the medulla and medullary rays, many of the lining cells contain numbers of wreath-like masses of granular and crystalline pigment. The adjoining vessels are injected with blood containing many pigmented leucocytes, apparently free pigment granules, and a few pigment wreaths, but no parasites.

Heart-muscle.—A considerable number of pigmented leucocytes are present in the capillaries but no parasites could be found.

Lungs contain a rich deposit of pigment lying principally in the capillary endothelium and outlining the vesicles in a pigment network. Some of these endothelial cells or macrophages are of extreme length and contain small wreaths of brownish pigment granules and crystals. Other cells show larger, denser clumps of pigment. In the pneumonic areas the exudate is composed largely of polynuclear leucocytes among which lie many pigmented cells.

Uterus.—The large superficial uterine sinuses contain blood-clots in many stages of organization. Over the inner surface of the uterus there is a thin

layer of pus and granular detritus entangling many pigmented leucocytes and a great many cocci in short chains. The evidences of exudative inflammation are limited to the superficial tissues. In the deeper tissue there is an extremely abundant deposit of wreath-shaped masses of pigment within capillaries, endothelial, large and small mononuclear, and connective-tissue cells. In many foci, especially in the neighborhood of large vessels, the pigment is quite as abundant as in the spleen. The sinuses and capillaries are injected with blood containing a large amount of free pigment and many pigmented leucocytes (Plate XI, Fig. 10). No malarial organisms could be identified. No smears were made from this organ.

Marrow.—The marrow sections and smears contain a moderate number of pigmented macrophages and endothelial cells. No infected red cells could be found. Nucleated red cells are abundant; the myelocytes are in excess; the giant cells are slightly increased; there are very few eosinophile cells.

Brain and Medulla.—A few pigmented endothelial cells and leucocytes were found, but no parasites.

Blood smears were made at the autopsy from the finger tip, mesenteric veins, and cerebral sinuses. The red cells exhibited the changes of a severe chlorotic anæmia with leucocytosis. A few pigmented leucocytes were seen. In spite of prolonged and repeated search no parasites could be identified in the blood smears.

The *blood-vessels* throughout many of the viscera contain an abundant deposit of pigment in granular and crystalline form, lying outside of and adherent to the red cells. Many of the red cells have a peculiar brownish tinge. Much pigment lies within leucocytes and endothelial cells, and the tissues in the neighborhood of such vessels sometimes contain a moderate deposit of pigment granules and crystals.

Epicritical:—The present case is inserted in this connection for the purpose of illustrating the great similarity which the pigment deposits of septic conditions may show to those of malaria.

In this case the possibility of malarial infection may be ruled out on the previous history, the distinct onset and course of ordinary septic metritis and peritonitis, by the failure of quinine to control the disease, and by the absence of parasites from the blood and viscera. On the other hand, the gross appearance of the spleen was identical with that of acute malarial infection, the liver and marrow exhibited a distinct brownish tinge, while the microscopic appearance of the pigment deposits was in many respects identical with that of malarial pigmentation.

On comparing these deposits with those of malaria some differences were to be noted. In the case of septicæmia the pigment grains were more often distinctly crystalline, and large, spheroidal, homogeneous black grains were more abundant than in acute malarial cases, while the fresh, yellowish, finely-granular pigment was much less abundant. The concentration of pigment deposits in the tissue about blood-vessels

was a somewhat characteristic feature. The wall of the uterus contained nearly as much pigment as the spleen. The pulmonary exudate contained an abundance of pigment, but in malaria pulmonary exudates have always been found nearly free from pigmented leucocytes. In the present case a considerable number of liver cells contained large and small vacuoles partly filled with brownish acicular crystals. In malaria this condition of the liver cells is rather rarely, though occasionally, encountered. In both cases the crystals fail to give Gmelin's test, they dissolve in ammonium sulphide, but not in ether, chloroform, or carbon bisulphide. The condition of the blood in sections of vessels showed that extensive destruction of blood cells was in progress throughout the viscera in the present case, and offers an ample source for the visceral deposits.

Nevertheless it would be impossible from a study of the pigment deposits alone to deny the coexistence of malarial infection, for as is shown in the description of other cases a similar destruction of blood may and in some degree frequently does accompany malarial infection. In Case VII the pigment deposit resulting from solution of blood cells completely overshadowed that derived from the malarial parasite.

The destruction of blood cells in various septic conditions may be confidently referred to the increased globulicidal action of the plasma which has long been known to characterize these conditions.

From the examination of this and many other cases I have become convinced that it is frequently impossible to distinguish between the pigment deposits of malaria and those resulting from increased globulicidal activity of the serum in other diseases. It follows that it is rarely, if ever, possible to establish the diagnosis of malaria from the presence of pigment deposits in the viscera. For in various other diseases there may be a deposition of pigment which is practically indistinguishable from that of malaria in color, form, intracellular position, general distribution, and chemical reactions. Moreover the slate color of the spleen in this case and the dark brownish tinge of the liver indicate that these are by no means pathognomonic gross signs of malarial infection. A further important inquiry concerns the extent to which the solution of blood cells in septic conditions is referable to post-mortem action of the globulicidal serum.

PART II.

I. TECHNICS.

Fixation:—The majority of investigators have preferred alcohol as a fixative agent in the study of tissues in malaria, placing small pieces in weak solutions for preliminary fixation, or using strong or absolute alcohol from the first. There seems to be nearly complete agreement that this is the best method to employ in this particular field. In addition to alcohol, a great variety of metallic solutions have been used with varying success, but none of them has been urgently recommended over alcohol.

In the present cases the fixatives used were alcohol, 80%, Lang's fluid, 1%, aqueous bichloride, and formalin 5-10%. The bichloride solutions may at once be discarded for the present purpose, on account of their tendency to leave metallic precipitates. In other respects they yielded good but not superior results. The tissues fixed in alcohol 80% gave satisfactory preservation of red cells and parasites, but less successful results as regards the tissues, which suffered the usual shrinkage. Hardening in formalin, 5-10%, proved most satisfactory. Shrinkage did not disturb the sections, the preservation of red cells and parasites was even better than that by alcohol, while the proved excellence of formalin as a fixative for general cytological study was very constantly apparent. Formalin seems to have a distinct advantage over some other fixatives in precipitating and rendering insoluble the pigments derived from dissolving red blood cells.

Staining:—Malarial parasites and their derivatives are well demonstrated in thin sections by staining 5 min. in 1% aqueous methylene blue, gently warmed, followed by moderate decolorization in strong alcohol, and clearing in oil of cajeput. This staining fluid may perhaps be slightly improved by adding a trace of alkali (.01% caustic potash), as used by Guarnieri. This method is practically that of Nissl and gives excellent demonstrations of changes in visceral as well as in ganglion cells.

Intracellular parasites are somewhat more deeply stained if the sections are previously treated for one hour with a moderately strong solution of hæmatoxylin, which also intensifies the nuclei of tissue cells. The use of hæmatoxylin clearly demonstrates the nuclei of the young and the segmenting parasites which are faintly or not at all stained in sections by methylene blue. *Amœba dysenteriæ* in sections stained by hæmatoxylin and methylene blue is very clearly demon-

strated, and may readily be identified by low magnification (Leitz No. 3). The nucleus of this protozoon fails to stain by methylene blue, but is sharply developed by hæmatoxylin. On several grounds, therefore, the combination of hæmatoxylin and methylene blue may be strongly recommended for the present purposes.

Attempts were made to stain the nucleus of the malarial parasite in tissues by the methods of Romanowsky, Ziemann, and Nocht. The results were not successful in demonstrating the chromatin, but Nocht's method brought out the body of the parasite very much better than any other method employed.

Very striking demonstrations of *Amœba dysenteriæ* were obtained by the application of this method to thin sections of the colon. These sections were stained for 24 hours and slightly decolorized 10-15 min. in 95% alcohol. The nucleus of the amœba then appears deep red, the body blue, the vacuoles are distinct and their contents variously stained.

In the study of pigment deposits in the viscera hæmosiderin was demonstrated by the common method, that of Perls. Sections were placed for one hour in 1% watery solution of potassium ferrocyanide and mounted in glycerine containing 1% hydrochloric acid. A useful procedure may sometimes be found in dissolving the malarial pigment by ammonium sulphide, which at the same time blackens the granular or diffuse hæmosiderin.

Formalin-fixation is a reliable means of identifying bilirubin, which is converted to the green biliverdin, but under the microscope the green tinge is not marked and the biliary derivatives may be mistaken for the yellow hæmosiderin granules.

I would strongly recommend the employment of smear preparations of the viscera, treated as blood specimens,[12] for the study of minute cellular changes, phagocytosis, and the demonstration of parasites. In the actively phagocytic viscera—liver, spleen, and marrow—it is often hazardous to attempt the identification of parasites in sections, whereas the examination of a smear made from these tissues furnishes convincing evidence of the number and type of parasites present.

II. THE VISCERAL LESIONS.

LIVER.

The *gross lesions* of the liver were usually but not always indicative of malarial infection. The organ was generally slightly swollen, but

[12] For the methods of staining smear specimens, see Ewing, Malarial parasitology, *Journal of Experimental Medicine*, 1901, v, p. 429.

this fact was not distinctly apparent at the autopsies, and in one case (III), with marked atrophy of liver cords and development of cavernous tissue, the liver was reduced in size. The consistence of the organ was usually slightly reduced. Fatty changes were seldom apparent to the naked eye. The pigment deposits were in the present cases always sufficient to give to the section of the organ a slightly brownish tint, but this change was sometimes far from characteristic and failed to be noted. In other cases the liver was slaty or black. The outlines of lobules were generally indistinct. Distension of the gall-bladder was commonly seen. That fatal malaria may leave inappreciable changes in the gross appearance of the liver is evident from Case VI, and especially from the cases of Marchiafava and Bignami in which very few or no parasites were found in the blood at death, and in which no microscopic evidences of a previous severe infection were found. It appears also that the pigmentation of the liver may be very slight when the parasites are massed in the intestinal mucosa.

The *microscopic examination* of the liver in the foregoing cases showed that the lesions varied but little from those described in acute malarial infection by Guarnieri in 1887, and Bignami in 1890.

Parasites, in the majority of cases, were comparatively scarce. They were usually englobed by phagocytic macrophages and endothelial cells, together with red cells, leucocytes, and pigment, and in these situations they almost invariably presented evidences of degeneration and solution. They were not positively identified in the liver cells. In Case II, dying just after sporulation of one of two or more very numerous broods of parasites, very large numbers of parasites were found in the liver, mostly within phagocytes and degenerating. In the other cases the hepatic phagocytes contained only scanty traces of the bodies of parasites, but many vacuoles and fresh pigment clumps. It is difficult to explain this difference except on the ground that the englobement of parasites in the liver is more active at certain periods of the cycle, or occurs intermittently, and that the destruction of englobed parasites may be completed very rapidly, i. e. within a few hours. Otherwise the livers of all acute cases with rich infection, ought to show abundant traces of the bodies of englobed parasites, which they did not show, with one exception, in this series of cases. It is to be noted that the exception was the only case of the series in which numerous rosettes were found in the tissues. There are several observations pointing to an increased phagocytic activity in the blood at the height of the paroxysm. Possibly the same rule holds in the liver.

However that may be, it is certain that the livers of acute cases with rich infection, exhibiting about an equal quantity of recent pigment, contain a very variable number of parasites in the early stages of destruction by phagocytes.

Among the active phagocytes could be identified the large mononuclear cells of the capillaries, endothelial cells, and leucocytes (Plate X, Figs. 1-6). There were often evidences of a fusion of large mononuclear cells, endothelial cells and leucocytes into large protoplasmic masses inclosing several infected red cells. Occlusion of capillaries by these masses or by many discrete cells was often observed, in the neighborhood of which the capillaries were sometimes found dilated.

Various lesions of the liver cells were observed. The normal liver cell stained by methylene blue, in sections and especially in smears, exhibits a coarse but regular and distinct chromatic reticulum. This reticulum was often found to be indistinct, its meshes uneven, and sometimes obliterated or displaced by various granules, indicating the changes of acute degeneration. Necrotic cells were rarely discovered in the present cases. I found many cells in advanced stages of degeneration, but few in which the nucleus was not demonstrable by hæmatoxylin or methylene blue, although the staining reaction was often faint.

Fatty infiltration of the liver cells was remarkably slight. In one case there were large areas in which the capillaries of irregular portions of lobules or of entire lobules were greatly dilated at the expense of the liver cords, which were markedly atrophic or had disappeared. The resulting appearance was that of cavernous tissue, as described in a few cases by Guarnieri, Bignami, Nepveu,[13] Lodigiani,[14] and Monti.[15] These dilated capillaries do not radiate from a central vein, as in the nutmeg liver of ordinary chronic congestion. Guarnieri refers this lesion to the disordered nutrition of liver and endothelial cells resulting from the obstructed circulation, partly also to direct pressure, conditions which existed prominently in my case. Bignami connects it especially with a previous necrosis of the liver cells. In the present cases these cells were not necrotic

The liver cells usually contained many large and small greenish granules of bile pigment. They were most abundant in Case VII, which exhibited externally the most intense jaundice, but were abundant in

[13] *Marseille médical*, 1894, xxxi, p. 649.

[14-15] Cited by Barbacci in *Centralbl. f. allg. Path. u path. Anat.*, 1899, x, p. 64.

Case II, in which the jaundice was slight. That the intercellular bile capillaries were extensively occluded by pressure of swollen endothelial cells and macrophages in distended capillaries was evident, and to this condition the biliary pigmentation of the liver cells may be referred.

In all cases the liver cells presented a variable number of fine, light yellow granules which gave the reactions of *hæmosiderin*, while a diffuse reaction for hæmosiderin was obtained in some endothelial cells and in the connective tissue of the portal canals.

I was unable to find evidences of the hyaline transformation of the nuclei of liver cells described by Guarnieri, except in a few isolated instances. A variety of nuclear changes were observed in the liver cells which did not differ from those seen in other infectious diseases. Many nuclei were found in the process of direct division, while mitotic figures were rare. In some cases the evidences of regeneration on the part of the liver cells seemed to justify the use of the term "diffuse vicarious hypertrophy," employed by Guarnieri.

The portal canals were sometimes markedly infiltrated with round cells. Mast-cells were rather numerous in some areas, and sometimes these cells were pigmented.

While the present cases do not bear directly upon the question of cirrhotic processes in the liver following malaria, it was strikingly apparent that the infection had exhausted itself in producing vascular and cellular alterations, while there was an entire absence of the changes of beginning fibrosis.

SPLEEN.

The spleen was increased in size in all cases, the change being generally proportionate with the length and, to a less extent, with the severity of the infection. It was noted at Montauk that the spleens of the malarial cases were as a rule much smaller than those of the typhoid cases. The organ was usually much softened and sometimes diffluent. The capsule was tense. In one case (VII) the spleen was rather firm and dark red. In the color there was usually distinct evidence of malarial infection, but this was sometimes slight, and the spleens of cases of typhoid fever in malarious subjects often gave no gross evidence of considerable pigment deposits demonstrable microscopically. In all the frank acute malarial cases the spleen was moderately enlarged, soft, and of dark brown, chocolate, or slate color.

The most prominent *microscopic* features were the pigment deposits and the cellular hyperplasia and distension of the pulp cords.

It was always difficult to identify positively parasites in the sections whereas they were always found in larger numbers in smears of the pulp tissue. They appeared to be more abundant than in the liver, and the same description of their inclosure and destruction in phagocytes applies to both liver and spleen. The macrophages were much more numerous and of larger size in the spleen than in the liver.

In all cases the sinuses and cords contained an excessive number of cells. The evidence of proliferation of splenic cells was most apparent in the frequent presence of islands of 8 to 10 cells of small size, compact grouping, deeply staining nuclei, and free from pigment. The Malpighian bodies participate largely in this process, as their dimensions were sometimes increased, and their outlines often irregular, and fringed at times with these islands of young cells.

Marked obstruction to the circulation in the spleen must have existed from the distension of sinuses by macrophages, swollen endothelial cells, leucocytes, and infected red cells. A pronounced condition of œdema was therefore nearly always present, and a few interstitial hæmorrhages, with necrosis of cells were found.

The deposit of pigment was usually more abundant in the spleen than in any other situation, and in acute cases was uniformly distributed in the macrophages, endothelial cells, and leucocytes of the pulp, while the Malpighian bodies were almost invariably free (Plate XIV, Fig. 16).

In the acute cases, the distribution of pigment was very uniform throughout the pulp. In a case of fatal typhoid fever which had been free from malarial paroxysms for three weeks, the pigment had been gathered in a network, with rather coarse meshes, throughout the pulp (Plate XIV, Fig. 17); while in another case of chronic malaria three months after the last acute seizure, the pulp was nearly free from pigment, which was gathered in large black intra- or extra-cellular blocks in the septa and walls of arterioles, and about the follicles (Plate XV, Fig. 18).

MARROW.

A chocolate tinge of the marrow expressed from ribs and vertebræ was a characteristic change observed in the acute cases, and, in general, this change in the marrow kept pace with the similar alteration of the spleen. In Cases I and II there was chocolate-colored marrow in the middle third of the humerus and clavicle, indicating an extensive increase in the natural limits of red marrow. Bignami demonstrated such a hyperplasia throughout the femur.

Of the changes in the marrow referable to the growth of parasites,

cellular hyperplasia, obstruction to the circulation, and deposit of pigment, it may be said that they are very similar to and of equal extent with those of the spleen. In Case IV there was an excessive accumulation of parasites in the marrow, the majority of red cells being infected with one or more rings, and multiple infection being very frequent. No infected nucleated red cells could be found.

Usually the number of parasites demonstrable in smears was moderate, though larger, as a rule, than in the liver or spleen. Crescents were not abundant in the tissues of any of the fatal cases, and when present they were not seen in unusual numbers in the marrow, as found by Councilman,[16] Marchiafava, Bastianelli and Bignami ('94), nor in the spleen, as stated by Bignami. In Case I, in which enormous numbers of crescents were found in the blood for two weeks, largely disappearing before death, there were very few crescents to be found in the marrow smears, and the pigment deposits in the marrow were comparatively scanty. This condition is not entirely in accord with the observations of the authors mentioned. In one case (VII) there were numerous small capillary hæmorrhages in the marrow.

Relation of lesions in marrow to malarial anæmia.—The chief interest in the lesions of the marrow in malaria lies in their relation to malarial anæmia, and in this field the studies of Bignami and Dionisi[17] are most complete. The changes in the blood and marrow in the writer's cases accord in a considerable degree with the classification given by these observers.

The most striking of the series in this regard is Case IV, in which there were the lesions of pernicious anæmia in the blood and marrow, and an excessive accumulation of parasites in the marrow. Besides the changes referable to the presence of many parasites and much pigment, the marrow showed well-marked cellular hyperplasia, leading to atrophy and disappearance of fat cells, which are normally present in the marrow of the vertebral bodies. In sections, this hyperplasia seemed to affect principally the large and small mononuclear cells, while in smears the new cells could be divided among the neutrophile myelocytes and the lymphocytes. These cells, together with macrophages and swollen endothelial cells, appeared to cause more than the usual obstruction to the circulation of the marrow, yet no necroses were discovered. The islands of nucleated red cells commonly seen in normal marrow were, in this case, entirely lacking in sections and smears.

[16] *Amer. Journ. Med. Sciences*, 1885, lxxxix, p. 416.

[17] Atti d. XI. Congr. med. internaz., Roma, 1894, ii, p. 235.

In their place there was a considerable number of megaloblasts with increased Hb. A peculiar abnormality, first noted in sections and fully verified in the smears, was the superabundance of giant cells, which occurred in groups of eight or ten. Bignami and Dionisi found this peculiarity in their fourth type of anæmia as occurring in malarial cachexia, and which was marked also by a condition termed by them sclerosis of the marrow. No changes to which the term sclerosis could be applied were found in the marrow in any of the present cases. The examination of the blood, showing the presence of a majority of megalocytes with increased Hb., and of many megaloblasts, together with the condition demonstrated by smears and sections of the marrow, indicates that the fetal type of blood formation had been established in this case, and warrants its classification as pernicious anæmia.

That such changes in the marrow are rather frequently initiated by malarial infection there can be no doubt, as shown by the pathological studies of Bignami and others. In my series of blood-examinations in malaria at Montauk and elsewhere there are no less than 19 cases in which pronounced features of primary pernicious anæmia were observed.

Some regard such evidence as demonstrating the lack of specific quality in the changes commonly regarded as pathognomonic of primary pernicious anæmia. Bignami, however, believes that these cases are still to be regarded as true examples of primary pernicious anæmia, claiming that the reversion of the marrow to the embryonal type of blood-formation, as seen in some cases of malarial anæmia, is not referable exclusively to the infection, but partly results from other associated causes not definitely understood. With this view I am in accord. The majority of cases of pernicious malaria develop the changes of secondary chlorotic or pernicious anæmia, but some show those of the primary pernicious anæmia. The essential difference is not in the cause but in the character of the anatomical changes in the bone marrow. In each instance the changes are initiated by malarial infection. In one case they are maintained almost exclusively by that infection, with which they are more or less proportionate, but in the other they are maintained by the peculiar changes in the marrow which when once initiated may progress independently. This view of the pathology of malarial anæmia, instead of weakening the evidence in favor of the specific nature of the changes in primary pernicious anæmia, furnishes, on the contrary, very strong proof of the specific quality of this condition of the blood.

The lesions found in the present cases, and in some others not reported in this series, indicate that the changes in the marrow in fatal cases of acute malaria follow one of two types:

(1) The cellular hyperplasia is pronounced, the nucleated red cells are abundant and tend to increase in size, the eosinophile cells, giant cells, and lymphocytes are over-abundant, while the fat cells are compressed and atrophic. With these changes, the blood shows moderate or severe anæmia of the chlorotic type, with a marked tendency to develop the signs of pernicious anæmia, which not infrequently become distinct. Pigment deposits and parasites are often unusually abundant as in Cases II and VI.

(2) The cellular hyperplasia is moderate, fat cells being abundant in the vertebræ and persisting in the ribs. Nucleated red blood cells, eosinophile cells, and giant-cells are deficient. The blood shows severe anæmia of chlorotic type. There may be considerable differences in the size of the red cells, but the Hb. is very deficient. The leucocytes are usually diminished, and eosinophile cells are scarce. Pigment deposits and parasites are usually not very abundant (Cases III and VI).

In attempting to draw these general distinctions it must be admitted that the present observations are too limited to fully establish general rules.

The natural limits of red marrow in the normal subject are rather variable, and the quantity of fat in the ribs and vertebræ decidedly so. Moreover, in a series of routine cases coming to autopsy from various causes I have found great dissimilarity in the appearance of the marrow cords, and of smears therefrom, which it is difficult to connect with the various states of nutrition and disease in the subjects. There will be no harm in describing the above types of changes in the marrow of pernicious malaria, if it is understood that it presents a tentative classification which is to be readjusted on the evidence of future investigations.

LEUCOCYTOSIS OF MALARIA.

Most observers have found very little change in the number of leucocytes in the finger blood during acute malarial attacks of average severity. This absence of leucocytosis with a rapidly rising temperature may be found of considerable corroborative value in the diagnosis of malarial fever.

A slight leucocytosis at the beginning of the paroxysm has been noted by Kelsch, Billings,[18] Vincent,[19] and others, but the numbers usually remain

[18] *Bulletin of the Johns Hopkins Hospital*, 1894, v, p. 89.

[19] *Annales de l'Institut Pasteur*, 1897, xi, p. 891.

below 10,000, while the percentage of polynuclear cells is increased. Vincent finds that quinine tends to increase the polynuclear leucocytes during the entire paroxysm. With the falling temperature and during apyrexia the leucocytes are usually distinctly diminished (2000-4000), especially the polynuclear forms, giving a relative lymphocytosis.

Except during the 3 to 4 hours immediately following the chill, therefore, malarial blood usually shows a diminished number of leucocytes, and a distinct relative lymphocytosis. The lymphocytes, small and large, may sometimes become quite numerous, especially in well-established cases. This fact accords with the increased cellular activity of the lymphoid tissues shown by microscopic examination of the viscera.

Bastianelli refers the loss of polynuclear leucocytes to the increased phagocytic activity of these cells. Vincent noted a periodical decrease in the number of large mononuclear cells, which he refered to the same process.

In the severer æstivo-autumnal paroxysms many observers have noted a distinct leucocytosis. Kelsch found that the leucocytosis of pernicious malarial attacks often consists in marked lymphocytosis. Bastianelli and Bignami find that in addition to various inflammatory complications, leucocytosis in pernicious malaria may result from rapidly progressive anæmia. They find it to be of frequent occurrence in hæmoglobinuric fever, and in cases attended with severe diarrhœa.

The presence of eosinophile cells may be noted in most cases of malarial fever, and these cells are usually increased in number during afebrile periods. Grawitz rightly regards this feature as of diagnostic importance, as in most diseases likely to be confused with malaria eosinophile cells are long absent or scarce. Bastianelli and Bignami found that eosinophile cells diminish during the paroxysm, and increase during apyrexia, while the blood is regenerating. In two cases of pernicious malaria, with many parasites, they found many mononuclear leucocytes and a very few eosinophile myelocytes, similar to those seen in myelogenous leukæmia.

In the present cases the usual behavior of the leucocytes was noted in the majority of instances.

In some severe and prolonged cases the lymphocytosis was a marked feature, these cells being distinctly increased in number. Eosinophile cells were never found greatly increased, but their nearly constant appearance in patients who were suffering from continuous or intermittent fever furnished a somewhat peculiar feature of the blood of malarial infection.

In some pernicious cases moderate polynuclear leucocytosis was observed; and in a few cases complicated by pneumonia, colitis, or severe cachexia, a considerable leucocytosis was found. In two of the fatal cases reported in this study, and in others, ante-mortem leucocytosis was observed, but the majority of fatal cases failed to show distinct leucocytosis. These estimates were all based on the examination of dry specimens.

Pigmented leucocytes were seen in the majority of cases, most abundantly in the severe and long-established fevers. They were found in nearly all fatal cases, but were most abundant in a case which recovered (No. 238). It appeared that pigmented leucocytes were more closely related to the severity of the antecedent paroxysms than to the extent of the pigment deposits in the viscera.

They were most abundant during and shortly after the febrile period, but were repeatedly found in afebrile cases and after parasites had disappeared from the blood. The phagocytic cells seen in the blood included mononuclear and polynuclear leucocytes and endothelial cells. The large and small mononuclear cells were most often found to contain pigment or parasites (Plate X, Fig. 6), but in a few cases, for reasons not clear, large numbers of polynuclear leucocytes were found harboring rosettes, other forms of parasites, and pigment (Plate X, Fig. 5). In a few cases very large endothelial macrophages were found in the blood containing parasites in all stages of degeneration (Plate X, Fig. 4).

The objects englobed by phagocytes as seen in the circulation included: (1) Parasites, free or inclosed in red cells; (2) Pigment elaborated by parasites, usually in small clumps, sometimes in large masses; (3) Hæmatoidin derived from the destruction of red cells; (4) Hæmosiderin derived from the detritus of red cells; (5) Intact or broken red cells; (6) Other leucocytes. Crystalline pigment was often seen in leucocytes in sections of tissues but never in the circulating blood during life. The degenerative changes in phagocytic leucocytes mentioned by Bastianelli and Bignami, including vacuolation and diminished staining capacity of nuclei, were noted in many severe cases. The number of vacuolated leucocytes (Plate XI, Fig. 8) found in the blood was always considerable and sometimes very large.

LYMPH NODES.

The abdominal lymph nodes were examined in all cases, and usually found moderately swollen, but always without signs of pigmentation. Microscopic examination was made in two cases only; in these the nodes were hyperplastic, the lymph sinuses were invisible, the stroma contained many mast-cells, and there was a scanty deposit of pigment, mostly in the endothelial cells.

LUNGS.

In gross appearance the lungs in pernicious malaria, both in these and in other reported cases, presented little that is characteristic of

the disease. They commonly contain a considerable deposit of pigment and are often very dark colored, but this feature is usually not distinctly characteristic, owing to the simultaneous presence of anthracotic pigment, hypostatic congestion, and frequently of jaundice.

Somewhat peculiar areas of lobular pneumonia were found in two cases, and have been described by Bignami, but the exudate was composed of the ordinary elements, and the inflammation was not specially connected with the growth of parasites.

Microscopically, the lungs are usually found to contain a very large number of pigmented cells, sifted from the general circulation, and in some cases a moderate additional deposit elaborated by the parasites in the pulmonary capillaries. Some large pulmonary macrophages, probably derived from other viscera, were found, filling a considerable length of the capillary. Thrombi composed of such cells, with pigmented leucocytes, swollen endothelium, and infected red cells were not infrequently found in the pulmonary capillaries of Case II. The lungs were œdematous but free from hepatization.

There appear to be no recorded instances of an excessive growth of parasites in the lungs comparable with that found in other viscera. Bignami speaks of a rusty color of the sputum in some cases of bronchitis in pernicious malaria, which he regards as of little clinical import, and I can find no report of the microscopic examination of such sputum, or of the lungs and bronchi in such cases, to show that an excessive growth of parasites in the lungs may give this character to the sputum.

From what has been shown of the action of the lungs in sifting bacteria and leucocytes from the blood in infectious diseases, it might be expected that the lungs would suffer severely in malarial infection. Their comparative immunity in this instance may perhaps be referred to the rapidity of the capillary circulation and the abundance of oxygen.

CARDIAC MUSCLE.

French writers especially (quoted by Laveran) have laid emphasis on the pallor and flaccidity of the myocardium in pernicious malaria. That condition was noted in nearly all the present cases at autopsy. In Case II nearly all tissues, including the heart muscle, were more or less discolored by malarial pigment. In Case VII, the heart muscle was generally light colored, but exhibited a slight brownish tinge referred at the time to the jaundice.

Microscopically, there were no pronounced changes found in the

muscle cells, but the perinuclear mass of large greenish granules was sometimes very abundant. Mast-cells were sometimes found in unusual numbers in the endomysium. Distinct fatty changes were not observed. With one exception, little pigment and few parasites were found in the capillaries. In Case II there was a notable exception to the usual rule and very large numbers of young parasites and pigmented cells were found completely filling distended capillaries throughout the heart wall (Plate X, Fig. 7). Although numerous parasites were found in other viscera and the brain was not examined, there was an excessive proportion in the heart muscle, while cerebral symptoms were late and cardiac failure was the most prominent clinical symptom.

Of similar cases, Benvenuti [20] has reported one in which the heart's capillaries were filled with infected red cells, the endothelium pigmented and degenerated, the muscle fibres swollen, their striation indistinct and the yellow pigment increased. In the brain it appears that there were as many parasites as in the heart, and many were also found in the kidney. Coma and cardiac dyspnœa were the principal symptoms. In another case Benvenuti found many parasites in brain, kidney, and heart, while the principal symptom was stupor. The clinical histories accompanying these reports are meagre for the present purpose, and it is difficult to judge of the relative number of parasites in the different viscera. I have been unable to find in the literature any other reports of such cases. Theobald Smith, however, finds that in Texas cattle fever very large numbers of parasites are commonly found in smears made from the heart muscle.

The available evidence, therefore, hardly seems to warrant a positive conclusion that acute cardiac failure in pernicious malaria may result from a massing of parasites in the cardiac muscle, and it would be safer to conclude from the present case merely that the condition of the cardiac and skeletal muscles demands more attention than is generally paid to these tissues in pathological studies of malaria.

KIDNEY.

Grossly, the kidneys in pernicious malaria usually give evidence of acute degeneration in their slightly increased size, diminished consistence, rather pale cortex, and indistinct but regular markings. There are usually no characteristic signs of malarial infection. In Case I the medulla and papillæ exhibited a somewhat peculiar darkening of color, from the unusual deposits of pigment. In some cases of black-

[20] *Policlinico*, 1896, iii–M., p. 390.

water fever the presence of extra-vascular blood in cortex and medulla considerably alters the above description, as in Case VII. In Case V the cortex was very light colored, while the medulla and papillæ were deep rust-colored from the large numbers of parasites in the capillaries in these areas. In this case there was also a large superficial anæmic infarct.

Microscopically, the usual lesions, well illustrated in the present cases, consist in granular, hydropic, and fatty degeneration, pigmentation by hæmosiderin granules, and sometimes isolated or diffuse necrosis of convoluted-tubule cells. The intertubular capillaries usually contain moderate numbers of pigmented leucocytes, macrophages, and infected red cells, while the glomeruli gather a larger number of similar elements.

The kidney rarely suffers from the accumulation of growing parasites, as does the brain, the mucous membranes, etc., a fact referred by Bignami to the rapid circulation in the organ. In blackwater fever I can find no reports of excessive numbers of parasites in the kidney, and in other cases, as a rule, the numbers of parasites have not exceeded those in the peripheral blood.

On the other hand, the eliminative function of the kidney exposes it to the effects of the toxæmia of malaria, so that albuminous urine is a very common clinical sign in acute pernicious malaria, especially if protracted, while the condition of the renal cells in the present cases, and in most others reported, shows nearly constantly a considerable damage to the organ from this cause. The lesions, however, were, in most of the present cases, of a purely degenerative type, without evidence of exudation into the stroma or other changes in the connective tissue. In Case III the degenerative changes were very intense, many cells were extensively eroded, and some necrotic, and although the tubules were dilated, and casts and granular coagulum were present in considerable abundance, indicating a near approach to exudative nephritis, yet there were no leucocytes in the tubules and no leucocytes or serum in the stroma.

With the minor exception of a slight glomerulitis, described in one case by Bignami, no more serious lesions have been found in the kidney in uncomplicated acute malaria. That the lesions are entirely out of proportion to the number of parasites in the organ and are probably of toxic origin has been generally accepted. The peculiar very abundant deposit of hæmosiderin granules in the renal cells, as illustrated in extreme degree in Cases III and VII, is a somewhat characteristic feature of the malarial kidney, but is sometimes seen in other diseases.

The anomalous condition of the medullary tubules in Case I, the lining cells containing enormous numbers of pigment wreaths, has already been considered in the report of that case (p. 124). Changes characteristic of "blackwater fever" were found in Case VII, and consisted in extreme congestion, numerous small hæmorrhages, excessive deposits of granular and crystalline pigment, probably derived from dissolved hæmoglobin, and peculiar necrosis of tubule-cells. In Case V the intense and peculiar degeneration of the tubule cells seems referable in part to the obstructed circulation from thrombi of infected red cells, while the hæmorrhagic type of the nephritis was distinctly the result of localization of parasites in the renal capillaries.

GASTRO-INTESTINAL TRACT.

In six of the present cases no gross lesions in the gastro-intestinal mucosa were to be found. In one case (III) considerable diarrhœa of long standing failed to leave any traces of inflammation in the intestine or colon. In Case VII an intense catarrhal colitis was apparently not caused directly by the malarial infection, as only moderate traces of parasites and pigment were found in the intestinal wall.

In Case VIII amœbic dysentery of moderate extent was combined with intense general malarial infection, but sections of the ulcers, while showing large numbers of the amœbæ, exhibited very scanty traces of malarial infection, no parasites and few pigmented leucocytes being found.

These cases indicate that marked diarrhœa in pernicious malaria may occur without anatomical changes in the intestinal mucosa, or may result from a secondary catarrhal colitis not directly caused by the malarial parasite, or may result from amœbic colitis, in which the malarial infection is not directly concerned. It has already been shown also that hæmatemesis, severe diarrhœa, and paroxysms resembling Asiatic cholera occur as the result of the massing of parasites in the gastro-intestinal mucosa.

According to Marchiafava, in the choleriform malarial cases, the parasites are very abundant in the mucosa of the small intestine, but scarce or absent elsewhere. Infected red cells may be identified in the stools, which are often bloody. The intestine contains bloody fluid and flocculi of mucus. The mucosa is swollen, congested, and shows superficial hæmorrhages and erosions. It is often dark brown or chocolate colored, while the unaffected light solitary follicles project prominently. Microscopic examination shows: (1) injection of vessels of mucosa and especially of villi with blood containing many parasites; (2) necrosis of epithelium of villi and mucosa, in which areas neither nuclei nor parasites can be stained; (3) infiltration with

leucocytes beneath the necrotic areas; (4) bacterial invasion of necrotic tissue; (5) mitotic division of nuclei of sound epithelial cells; (6) freedom from parasites of vessels of submucosa, which contain many pigmented leucocytes.

In Case II, although a considerable number of parasites was found in the intestinal mucosa, there were no changes referable to their presence, and the patient did not suffer from diarrhœa.

There has apparently been little opportunity to ascertain the character of reparative processes which may follow the acute lesions in choleriform pernicious malaria, since such cases are usually fatal. Pensuti,[21] however, has furnished one observation of interest in this connection. A patient who had suffered in November, 1892, from severe malaria with vomiting and profuse diarrhœa, was treated actively by quinine, but the diarrhœa persisted and he died of broncho-pneumonia in February, 1893. In the intestine, especially in the ileum, the mucosa was hyperæmic, and showed some amyloid changes in the vessels. In many places there was complete disappearance of the glandular layer, which was replaced by young connective tissue. The remaining islands of glandular tissue showed marked hypertrophy of alveoli. A good deal of pigment (character not stated) was found in the mucosa. Pensuti regarded the lesion as referable to the toxic effects of malaria.

There seems to be little pathological evidence on which to discuss the relation to malaria of some forms of tropical colitis not amœbic, but often associated with pernicious malaria.

CENTRAL NERVOUS SYSTEM.

Gross appearances.—In some cases of comatose malaria with rich infection the brain presents a characteristic brownish discoloration, most marked in the gray matter, which results from deposits of pigment. This condition has been found in a considerable number of cases, but by no means in all. In 10 cases Kelsch and Kiener found marked discoloration of the cortex in 9, and faint changes in 11 cases. When present it invariably indicates the presence of a very large number of parasites and much pigment in the brain tissue. Its absence, on the other hand, by no means excludes the presence of a large number of pigment-free parasites in the gray matter, a fact which was apparent in some of my cases not reported here, and which is referable to several causes. The æstivo-autumnal parasite is not always a very active pigment-producer, and Guarnieri and Bignami have depicted cerebral capillaries completely thrombosed by pigment-free rosettes. Very large

[21] *Gazz. med. di Roma*, 1893, xix, p. 121.

numbers of young parasites may therefore be present in a tissue which shows very little gross evidence of pigmentation. The discoloration resulting from jaundice, which frequently complicates fatal malaria, may obscure the effects of malarial pigmentation. Finally, as will subsequently be shown, the majority of cases of comatose malaria do not exhibit a massing of parasites and pigment in the brain, so that the characteristic discoloration of the gray matter sometimes found is not to be expected in these cases. Accordingly of eight cases of comatose pernicious malaria in which I was able to examine the brain a brownish discoloration, which could be regarded as absolutely characteristic of malaria, was not found in any. Most of the cases were jaundiced, but in two others showing many parasites the discoloration present was too faint to be regarded as pathognomonic of the disease.

Multiple hæmorrhages in the gray matter have been described in comatose malaria by Guarnieri, Bignami, Marchoux,[22] Monti, Bastianelli, Blanc, and Spiller.[23] These lesions were not discovered in any of my cases. In Bastianelli's case the hæmorrhages were limited to the cerebellum, while disturbances of equilibrium were said to have been prominent symptoms during life. In a case reported by Blanc, in addition to numerous capillary hæmorrhages, there was a large subcortical clot.

Pial œdema of moderate grade has been found in the majority of cases, but cannot be regarded as of special significance.

Blanc and Borrut claim to have observed true inflammatory exudative lesions in the brain in pernicious malaria, but their observations have not been confirmed. Maillot, in 1851, mentioned two cases of red softening of the lower dorsal cord.

Microscopic changes.—The microscopic appearances of the brain tissue in the typical cases of pernicious malaria of cerebral type are too well known to warrant minute description here. The principal feature is the massing of red cells infected with various forms of æstivo-autumnal parasites in the capillaries. Usually the parasites have been found uniformly distributed in the brain and cord, but Marchiafava[24] observed a case with bulbar symptoms, in which there was special localization in the medulla, and in a case of Bastianelli's the limitation of hæmorrhages to the cerebellum indicated a special massing of parasites in that region. The numbers of these parasites are sometimes

[22] *Annales de l'Institut Pasteur*, 1897, xi, p. 640.

[23] *Amer. Journ. Med. Sciences*, 1900, cxx, p. 629.

[24] *Lavori d. III. Congr. di med. int.*, Roma, 1890, p. 142.

enormous, often partly or completely occluding the lumen of the vessel. Complete thrombosis frequently results from the agglutination of infected red cells, pigmented leucocytes, and swollen endothelial cells. Capillary hæmorrhages result in the neighborhood of such thrombi, and are probably preceded by degenerative changes in the capillary endothelium. While most of the fixed pigment is found in the endothelium and in circumvascular lymph spaces, parasites are rarely seen in the endothelial cells. Monti, however, describes well preserved parasites in degenerating endothelia and believes that the parasites are sometimes capable of development in these cells. Such an occurrence is at least unusual.

To the general condition of obstructed circulation it is probably safe to refer the marked cerebral symptoms of such cases of acute pernicious malaria.

The infiltration of pericellular lymph spaces with small round cells, which is seen in many infectious diseases, was noted in some of the present cases, but not in excessive degree.

The deposit about the ganglion cells of peculiar masses of variously twisted threads and rods staining densely with methylene blue, was noted in the description of Case II.

Considerable attention has been paid in recent years to the condition of the *ganglion cells* in comatose cases of malaria. Monti studied the changes in the ganglion cells in several cases by means of Golgi's method. In some instances no important alterations of the ganglion cells were found. In others, with severe nervous symptoms, extensive changes were discovered, of focal distribution, affecting principally the dendrites. These processes were thinned in places and beset with many small swellings, and the changes affected either the finest twigs only, or the entire dendritic system, or the cell body itself was shrunken and irregular. Some dendrites showed the usual changes of varicose atrophy. The axis cylinder processes were sometimes found normal, but in the severe comatose cases extensive changes of the above types affected the axis cylinder as well as the dendritic processes. Monti referred the changes to occlusion of capillaries, finding them very similar to the lesions produced by multiple emboli produced by intravascular injection of lycopodium.

The lesions in the ganglion cells demonstrated by Nissl's method have appeared in the present cases not to differ essentially from those seen in other infectious diseases with marked cerebral symptoms. These changes consist principally in varying degrees of chromatolysis

affecting the cortical cells rather uniformly, but the cells of the bulbar nuclei more irregularly. The earlier stages of the lesions include reduction in size, irregularity and subdivision of the chromatic bodies, usually beginning in the dendrites, later involving the cell body. In more advanced stages the chromatic bodies are largely destroyed, and a moderate number of cells may be entirely bereft of chromatic substance. The more serious lesions of true acute degeneration, such as destruction of cyto-reticulum, shrinkage and cleavage of cell body, vacuolation, and nuclear changes, were seldom seen in my cases.

As to causation of these lesions, probably local disturbances of circulation are a more important factor in malaria than in most other diseases, but the comparative uniformity of the lesions noted indicates that a general toxæmia is, even in cerebral cases, the more important pathogenic agent.

A great variety of *nervous symptoms* referable to disturbances of the central nervous system have been attributed to malarial infection by early writers whose reports lack the evidence, now demanded, of a positive blood examination. The rather extensive literature of this subject has been fully considered by Mannaberg, and in a recent article by Bardellini.[25]

Since the discovery of the parasite, there is good clinical and in some cases anatomical evidence indicating that many of the nervous symptoms early referred to malaria may really be dependent on mechanical or toxic lesions resulting from this infection. Authentic cases have been reported showing hemiplegia (Marchiafava, Vespa); general convulsions (Marchiafava, Bignami, Baccelli); tetanic spasms after acute malaria (De Francesco); athetoid movements (Boinet and Salebert); disturbances of equilibrium (Bastianelli, Bignami); trismus, nystagmus, various toxic and clonic spasms (by many writers); post-malarial psychical disturbances (Pasmanik, Ségard, and many others); symptoms of disseminated sclerosis, paresis, increased reflexes, ataxia, bulbar paresis, in two cases (Angelini and Torti); electric chorea of Dubini (Bastianelli and Bignami); paralysis of the bladder from spinal lesions (Bardellini); hyperidrosis from affection of the sympathetic (Bardellini); and polyneuritis somewhat resembling Landry's paralysis (Bardellini, Torti, Mesnard). Bardellini concludes that when nervous lesions are transitory they are probably embolic, but when permanent they are probably complicated by multiple hæmorrhages. In a review of the recent literature he could find no satisfactory evidence that periodic neuralgias may be directly referable to malarial infection.

It must be admitted that while the etiology in most of the above cases was probably malarial, yet the evidence is generally unsatisfactory and, usually lacking anatomical support, is inconclusive, while in some instances the malarial infection was clearly secondary and had nothing to do with the chronic lesions.

[25] *Annali di medicina navale*, 1898, iv, p. 919.

Coma in pernicious malaria.—From the study of 64 cases of malarial coma at Montauk and in New York, some of which are included in the present series, it appears that this cerebral symptom in malaria occurs in three rather distinct clinical pictures and under three entirely different pathological conditions.

(1) *Malarial coma may be referable to massing of young amœboid parasites in the cerebral capillaries.*

This type of coma, which has long been recognized as a frequent form of pernicious malaria, is illustrated in Case III in which the cerebral symptoms were found to be associated with an extensive massing of parasites in the cerebral capillaries, while the deepening stages of coma could apparently be connected with the increase in size of the parasites and the gradual filling of the vessels with thrombi of infected red cells, pigmented leucocytes, and swollen endothelial cells. No capillary hæmorrhages were discovered.

Clinically, the coma resulting from this pathological condition is rather slowly established in the course of active infections, when many young parasites are found in the finger blood and when the temperature is elevated. The patient is usually first delirious, then mildly comatose, then deeply comatose, finally stuporous, with abolition of pupillary and other reflexes, and almost always dies within 48 hours after the beginning of marked cerebral symptoms. Of 11 such cases observed at Montauk 10 died, and very vigorous treatment succeeded in saving only one.

(2) *Malarial coma may be referable to embolic processes with temporary occlusion of vessels in small areas of the brain, and without uniform massing of parasites in cerebral capillaries.*

In these cases the coma develops suddenly and may be as suddenly recovered from. In a case previously reported the patient three times in five days fell back unconscious in bed, his pipe dropping from his mouth, but after a variable period he recovered consciousness, picked up his pipe and resumed smoking. From this very transient form the duration of the coma may be much more prolonged and serious, but it is seldom fatal. It may occur in febrile or afebrile cases and may exhibit distinct symptoms of focal irritation or meningitis. In the blood, few or many crescents, sometimes tertian parasites, but very few rings are usually found, and occasionally no parasites can be discovered. Emboli of parasites, pigmented leucocytes, and visceral macrophages, seems to be the only anatomical lesion which can explain such symptoms. They arise in established cases of the disease and

on microscopic examination extensive malarial lesions are found in the viscera but few or no parasites are to be found in the brain. Although crescents or tertian parasites may be abundant in the peripheral blood in these cases, I have not seen, nor been able to find in the literature report of any case in which large numbers of crescents or tertian parasites were found occluding cerebral vessels, and it appears that these parasites do not exhibit the tendency to unequal distribution in any degree comparable with the fertile æstivo-autumnal forms.

(3) *Malarial coma may be referable to the general toxæmia of the infection.*

In these cases the coma usually develops slowly but may in cachectic cases be ushered in suddenly, apparently by some embolic process. It is often of prolonged duration and not being caused by massing of young parasites in cerebral vessels it is unaffected by quinine. Occurring only in severe cases and being associated with serious toxic lesions in many viscera it is nearly always fatal.

Cases I and IV of the present series illustrate this type of coma. These patients were comatose, one at least three days and the other for two weeks before death. As no other cause for the coma was found it had to be referred to the malarial infection, which was very severe and long established. These cases differed radically from the classical type of comatose malaria, as in one only a few crescents, and in the other only tertian parasites, were present in the blood, and no parasites and comparatively little pigment were found in the brains. They show conclusively that the coma of pernicious malaria is not always referable to the presence of parasites in the cerebral capillaries. In the Montauk series[26] there were four other fatal cases of comatose æstivo-autumnal malaria in which crescents only were found in the blood, and one other fatal tertian case with prolonged coma. Jancsó and Rosenberger[27] also have reported a fatal comatose case in which the brain contained few parasites, which were abundant in the other viscera, the coma being referred by the authors to a toxic origin. It is possible that too little attention has been paid to the opinion of Guarnieri that malarial coma may sometimes be caused by obstruction to the portal circulation. This opinion was based upon the evident obstruction to the hepatic circulation commonly found in the liver of pernicious malaria, and upon the experimental production in dogs of coma without convulsions by ligature of the portal vein.

[26] Ewing, *N. Y. Med. Jour.*, 1899, lxix, pp. 114; 149.

[27] *Deutsches Arch. f. klin. Med.*, 1896, lvii, p. 449.

Uræmia may possibly be held partly responsible for some cases of this type but the clinical picture is not that of uræmia and the renal changes are not such as are commonly associated with uræmia.

Distinct differences in prognosis belong to these three varieties of coma.

Of eleven cases in which coma supervened during the development of a numerous brood of parasites, ten were fatal. Some of these, but not all, were of the classical cerebral type with massing of parasites in the brain. The energetic use of quinine has some influence in such cases.

When coma develops gradually in severe and long established cases, with few young parasites in the blood, it is usually of toxic origin, is unaffected by quinine, and is almost invariably fatal.

Of 33 cases of coma developing, often suddenly, in cases with crescents only in the blood, there were but three fatalities.

THE MALARIAL PIGMENTS.

Two forms of malarial pigment have long been recognized. One of these, melanin, is granular, brownish, elaborated directly by the parasite, according to Sacharoff from the nuclear remnants in the red cell, and fails to give the Prussian blue reaction of hæmosiderin. It is not, on that account, necessarily free from iron. This pigment is dissolved by ammonium sulphide and readily by heat, but long resists the action of strong acids and alkalis, and I have found it to be insoluble in hardened tissues by chloroform, ether, or carbon bisulphide.

The other described form of malarial pigment occurs more abundantly in protracted cases, as small yellowish granules, located principally in the tissue cells, especially in the liver, kidney, spleen, and marrow. It yields, when fresh, the reaction of Prussian blue, but gradually loses this reaction. It is probably the hydrated oxide of iron (Thoma).

When the Hb. of red cells is dissolved in the plasma, as occurs in poisoning by potassium chlorate, arsenic, etc., and in diseases such as pernicious anæmia, scorbutic ailments, malaria, septicæmia, etc., it may be found in the tissues in granular or crystalline form and of dark brown or reddish color. Sometimes such Hb. granules are soluble in water, but more often they are altered in some way and become insoluble in water, when they have been called "parahæmoglobin" by Neucki. Parahæmoglobin is very nearly identical with hæmatoidin, which is frequently precipitated in granular or crystalline form from blood extra-

vasations, and both are soluble in chloroform and carbon bisulphide. Perls, Thoma, and Ziegler, on whose authority these statements are made, leave one to infer that since melanin, the black malarial pigment, is insoluble in chloroform, etc., this test furnishes a chemical reaction which may distinguish hæmatoidin from malarial pigment. On submitting formalin-hardened tissues containing old blood extravasations to the action of chloroform, I find that two weeks' exposure has no effect upon the granules and crystals of hæmatoidin. Possibly they had been altered by age, or by the action of formalin, but the same result was obtained in tissues hardened in alcohol. Both hæmatoidin and black malarial pigment were dissolved by ammonium sulphide. Accordingly I failed to find the described chemical reactions of hæmatoidin of practical value, at least in hardened tissues, in distinguishing between malarial pigment and the granular and crystalline deposits derived from destruction of red blood cells.

Another difficulty arises in the identification of malarial pigment in tissues. Many cases of pernicious malaria are attended with marked jaundice. In many cases of jaundice from other causes bilirubin, now regarded as identical with hæmatoidin, is deposited in granular or more often in crystalline form, and is identical in color with hæmatoidin and with much pigment found in the bodies of malarial parasites. In sections of two cases of marked jaundice following pneumonia, one in a young infant, the other in an adult, I found many crystals and granules of bilirubin (?) (Perls, Ziegler) in the hepatic endothelium. In the case of the infant, they were found in large masses in the liver cells also, as well as in many leucocytes in various organs, which very closely resembled the pigmented leücocytes of malaria. Now bilirubin in fresh bile is turned green by formalin and gives Gmelin's reaction, but the crystals in neither of these cases turned green in formalin nor gave Gmelin's reaction. There were, however, other greenish particles in the formalin-hardened sections of both livers. In both cases chloroform failed to alter the crystals in two weeks, and they failed to give the Prussian blue reaction. That the crystalline deposits in the liver cells of the new-born infant were derived from the bile there can be little doubt, and the failure of the reactions may be explained, as is done by Gerhardt and others (quoted by Perls), who find that in many forms of jaundice urobilin and not bilirubin is formed. Urobilin fails to give Gmelin's reaction. Formalin-hardened sections of these tissues were treated for 24 hours, also, in carbon bisulphide, and in ether, but no change in the pigment was observed. The same negative result was obtained with sections of malarial tissues.

These cases are briefly referred to in order to point out that the jaundice of infectious diseases may cause deposits of pigment which are indistinguishable morphologically, and by all ordinary chemical procedures, from much malarial pigment. Various reports of the finding of malarial pigment in the liver cells in pernicious malaria have possibly not been made with full recognition of this fact. The positive identification of malarial pigment therefore becomes a matter of great difficulty, for it appears from the above considerations that in malarial fever one may meet with granular, sometimes crystalline pigment particles, free in the vessels or englobed in various cells, not giving the Prussian blue reaction, nor dissolving in chloroform, ether, or carbon bisulphide, but dissolving in ammonium sulphide, which may have any one of the following origins:

(1) Pigment elaborated by the intracellular parasite.

(2) Hæmatoidin derived from the remnants of infected red cells.

(3) Hæmatoidin or altered hæmoglobin deposited in granular or crystalline form from red cells dissolved in the plasma. (Hæmoglobinuric fever, jaundice, extravasated blood, post-mortem processes.)

(4) Bilirubin or urobilin granules or crystals.

Throughout the study of the present cases, in addition to the dark brownish granular pigment inclosed in cells or parasites, larger dark brown particles exhibiting more or less crystalline forms were frequently found. When scanty they were usually limited to the spleen, but in Case VII there were excessive deposits in all viscera except the liver and spleen. Since the pigment in parasites is invariably granular and not crystalline—possibly there are rare exceptions in the crescentic bodies—the above considerations render it extremely probable that the strictly malarial pigment, i. e., that derived from parasites, is never found in crystalline form, and that all such crystals ought to be referred to some other origin. Moreover as the detritus from dissolved red cells may be found as brownish granules in the bodies of phagocytes, it cannot be claimed, even for the pigmented macrophages, that all granular pigment is derived from parasites.

When one follows the changes that occur in degenerating parasites and red cells within a macrophage, there are, however, certain long-retained characters which often serve to distinguish the pigment derived from parasites from that resulting from the destruction of red cells.

In sections of tissues, the pigment within parasites is invariably finely granular, first appearing as one or two fine grains, later as a

larger, more or less irregular clump, while in æstivo-autumnal rosettes the pigment appears in a rather compact spheroidal mass of fine granules. In sections, the pigment is usually less compact than in blood smears, in which it may often be found in a single large block with outlying grains. When these pigmented parasites are englobed in macrophages the body of the parasite rapidly disappears, in 4 to 5 hours according to Marchiafava, leaving a small vacuole about the pigment clump, and usually leaving the pigment undisturbed in arrangement of granules for some days. When the pigment clump is englobed after its discharge from the rosette into the plasma, it appears sometimes to retain its compact arrangement, in which case the englobed mass fails to show a surrounding vacuole. In the leucocytes of the peripheral blood, which appear to absorb most of their pigment from the plasma, it is seldom possible to detect any vacuole surrounding the clump. When a red cell is englobed in a macrophage, the Hb. is frequently reduced to brownish granules skirting the periphery of the space originally occupied by the red cell, or sometimes showing a less regular arrangement of fine granules within a large vacuole.

Finally, when a red cell infected by a pigmented parasite is englobed, the destruction of the red cells may leave a peripheral ring of granules, while the larger compact mass from the parasite occupies a central or peripheral position (Plate X, Figs. 1 and 4).

From a minute study of the appearances of intracellular pigment clumps in sections, and especially in smears of tissues, it is possible to follow degenerating parasites and red cells through all the stages just described, and to distinguish in many instances pigment derived from parasites from that resulting from the destruction of red cells. After a variable time all englobed pigment appears to concentrate in more compact perinuclear masses, and the above features can no longer be identified. Some of the larger masses appear to form by the coalescence of two or more vacuoles.

When one compares the deposits of hæmoglobin which occur in inflamed tissues infiltrated with blood, with deposits of malarial pigment, certain characters are often distinctly apparent which serve to distinguish the one from other. Chief among these is the crystalline form of much of the pigment in the inflammatory deposits. In many such cases the formation of intra- and extra-cellular circles of crystalline pigment may be traced from whole or subdivided red cells. Such features were noted especially in the renal tubules of Case I of the present series (Plate XI, Fig. 9). Sometimes the pigment circle repro-

duces the original size of the intact red cell, usually it is smaller, the red cell having been subdivided before its final alteration, while frequently the crystals are isolated and elongated, or of small size resembling granules. No spheroidal clumps of finely granular pigment like that of the æstivo-autumnal rosette were seen in several inflamed mucous membranes with disintegrating blood, examined for this purpose. In cases of pneumonia, septicæmia, and blood extravasations from various causes, along with the crystalline pigment there was much coarsely granular pigment in leucocytes and endothelial cells, but none or very few of the clumps exhibited the finely granular character of fresh malarial pigment.

Since malarial pigment as seen in the parasite is practically never found in crystalline form, and there seems to be no good reason to assume its transformation into crystals after englobement by phagocytes, crystalline pigment must be referred to other sources, principally the destruction of red cells.

According to Thoma, hæmosiderin probably results when the detritus of red cells is acted upon by living tissue cells, while hæmatoidin is produced in the bulk of a blood extravasation where living phagocytes are absent.

The study of hepatic macrophages in malaria shows, however, that englobed red cells may be reduced to hæmatoidin as well as to hæmosiderin. Not infrequently one finds in the bodies of such macrophages red cells which give the hæmosiderin reaction, but show in addition some black hæmatoidin grains. This fact has also been noted by Barker. The two pigments appear to form, in malaria, under nearly identical conditions, and in the large macrophages it appears certain that the majority of englobed red cells are reduced to hæmatoidin.

Not having found in chloroform, ether, carbon bisulphide, acidified potassium ferrocyanide, or ammonium sulphide, any chemical reagent that will distinguish malarial pigment from parahæmoglobin, hæmatoidin, bilirubin, or urobilin, etc., one must apparently rely upon morphology alone for its identification in hardened specimens.

In pernicious malaria, the formation of pigment by the parasite, the intracellular destruction of red cells, the solution of Hb. in the plasma, occurring most extensively in hæmoglobinuric fever, but seen to some extent probably in all fatal cases, the deposit of bilirubin or urobilin crystals or granules in jaundiced cases, all are processes which are variously intermingled in the disease, and in the study of the tissues it is of prime importance (see Cases I and VII) to distinguish as far as possible between them.

Case X has been reported here in order to illustrate the practical importance of the foregoing observations.

Certain alterations of interest were noted in the deposits of pigment in liver and spleen after subsidence of the infection. Throughout the periods of active infection the pigment was richly and uniformly distributed in all parts of the organs. In a case of typhoid fever in a malarious subject dying from perforation three weeks after the disappearance of parasites under quinine, the pigment in the liver was found in less numerous but larger and more compact clumps within the capillaries, but very little had yet reached the portal canals. In the spleen the beginning concentration of pigment had caused the appearance of a pigment network with large meshes, within which were newly-formed cells free from pigment (Plate XIV, Fig. 17).

In another case, dying three months after subsidence of the infection, the greater concentration of pigment is clearly indicated in the photograph (Plate XV, Fig. 18).

Ferrier,[28] in a case dying one month after cessation of fever, found a moderate amount of pigment in the cells of the pulp cords only. In another case, after a similar period, much pigment in nodular blocks 2-3 times the diameter of the splenic cells was found in the splenic cords. Rather less pigment was found in the arterial walls and peripheries of follicles. In a third case, six weeks after acute symptoms, most of the pigment was found in very large cells in the centres of the pulp cords.

DESCRIPTION OF PLATES X-XV.

PLATE X.

Fig. 1. Appearance of hepatic macrophage in Case II. Various stages of destruction of infected and uninfected red cells and of parasites.

Fig. 2. Hepatic endothelial cell in established æstivo-autumnal fever, containing pigment.

Fig. 3. Hepatic macrophage in Case VII, fatal infection with large tertian parasite. The pigment is distributed in fine granules and in blocks.

Fig. 4. Endothelial macrophage of circulating blood, containing rings and masses of pigment and degenerating parasites. Red blood corpuscle at the side.

Fig. 5. Polynuclear leucocyte of circulating blood with englobed rosette and full grown parasite.

Fig. 6. The usual pigmented mononuclear leucocyte of the circulating blood.

Fig. 7. Capillary in muscle of the heart, showing numerous intra-corpuscular parasites. Case II.

PLATE XI.

Fig. 8. Appearance when stained of vacuolated, pigmented leucocyte, common in malarial blood.

[28] *Arch. de méd. expér.*, 1897, ix, p. 87.

Fig. 9. Pigmentary deposits in cells of renal tubule, from disintegrating red corpuscles. Case I.

Fig. 10. From section of uterine submucosa of Case X, puerperal septicæmia. Deposits of wreaths of granular and crystalline pigment, resembling malarial pigment, in tissue infiltrated with blood.

PLATE XII.

Fig. 11. Early stages of destruction of red corpuscles with deposit of peripheral rings of hæmatoidin. Pigmented leucocytes. Case VII and others.

Fig. 12. Later stages of destruction of red corpuscles with deposit of crystals of hæmatoidin. Case VII.

PLATE XIII.

Fig. 13. Photograph of section of kidney, Case V. Degeneration of renal epithelium and granular coagulum around glomerulus. × 250.

Fig. 14. Photograph of section of kidney, Case V, showing massing of malarial parasites in renal capillaries. × 250.

Fig. 15. Photograph of section × 1000, same case, showing pigmented parasites in renal capillary.

PLATE XIV.

Fig. 16. Photograph of section of spleen, Case VII, showing distribution of pigment in acute pernicious malaria.

Fig. 17. Photograph showing reticular arrangement of pigment in the spleen, three weeks after subsidence of acute malarial infection.

PLATE XV.

Fig. 18. Photograph showing disposition of pigment in the spleen, three months after acute malarial infection. Case IX.

Fig. 19. Photograph showing distribution of pigment in the liver, three months after acute malarial infection. Case IX.

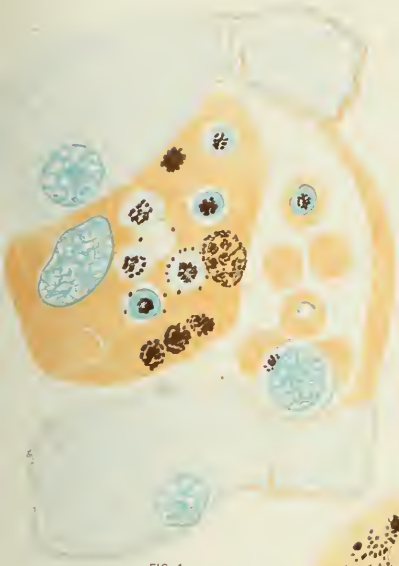


FIG. 1.

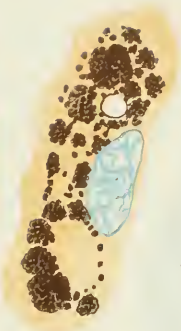


FIG. 2.

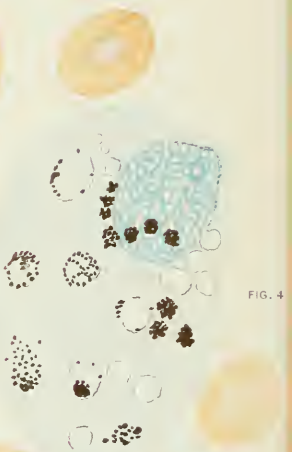


FIG. 4

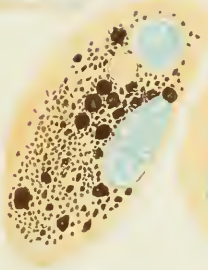


FIG. 3.



FIG. 5.



FIG. 6.

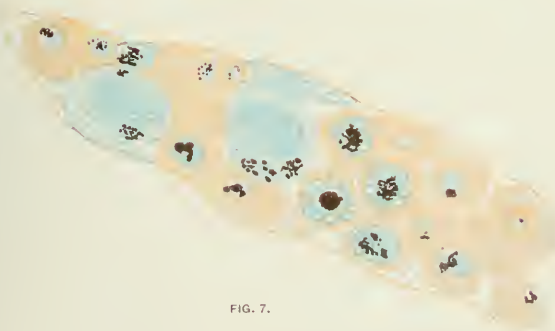


FIG. 7.

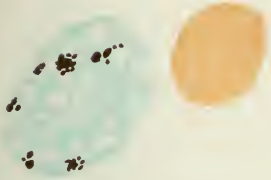


FIG. 8.

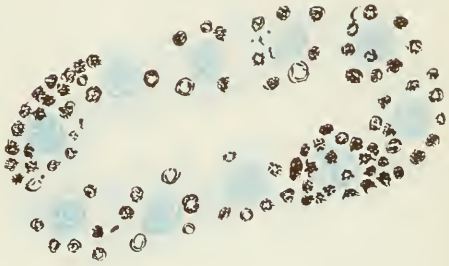


FIG. 9.

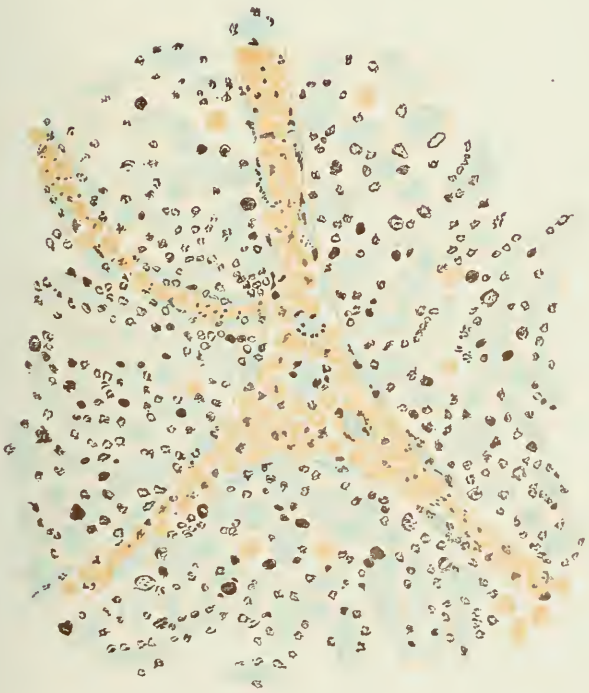


FIG. 10.

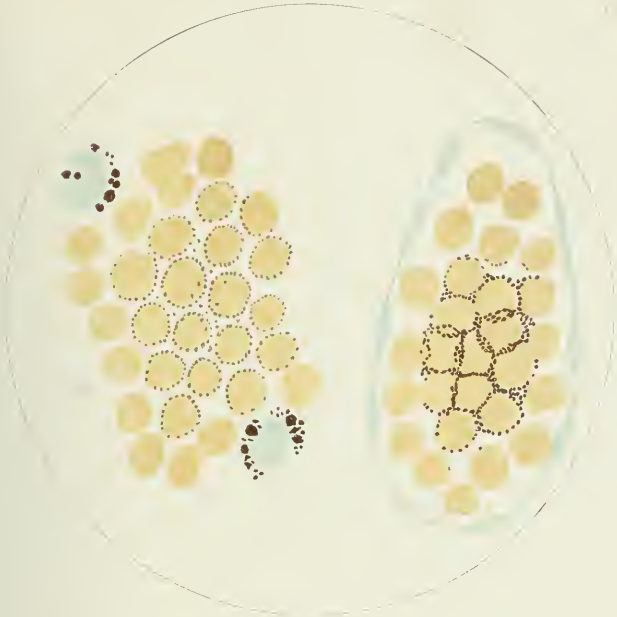


FIG. 11.



FIG. 12.

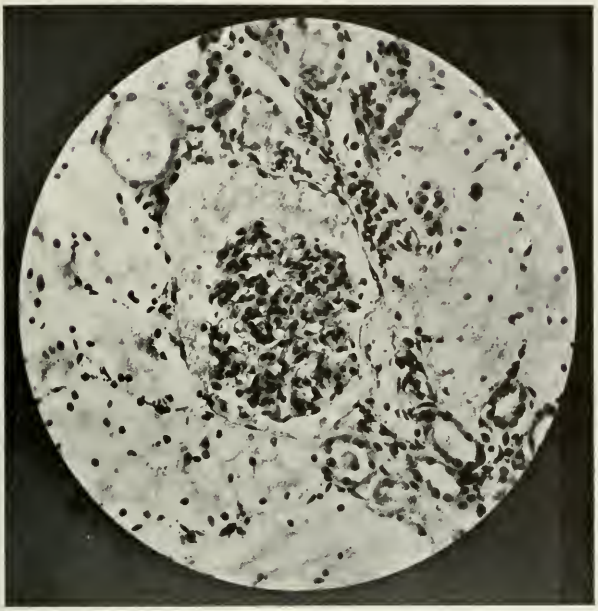


FIG. 13.

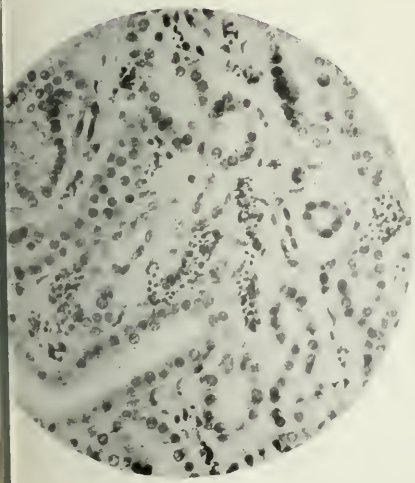


FIG. 14.

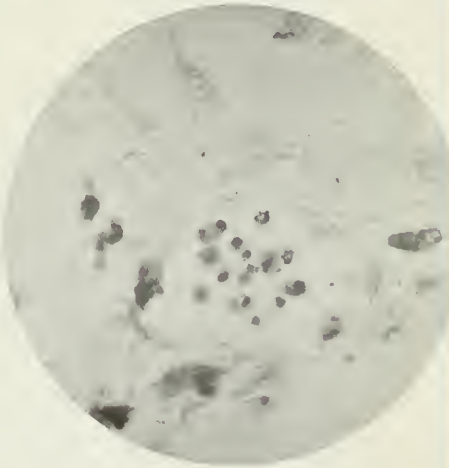


FIG. 15.

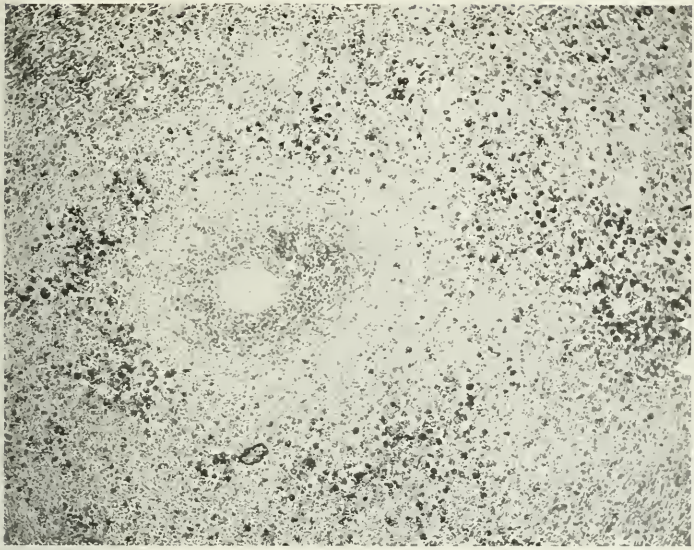


FIG. 16.

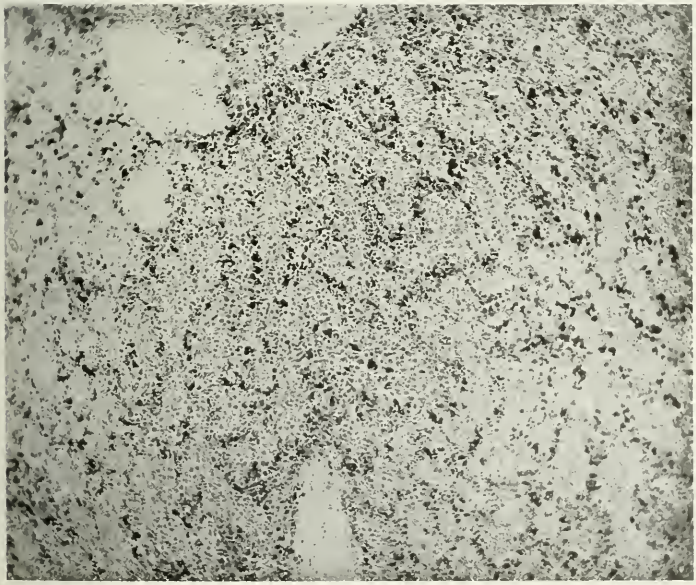


FIG. 17.

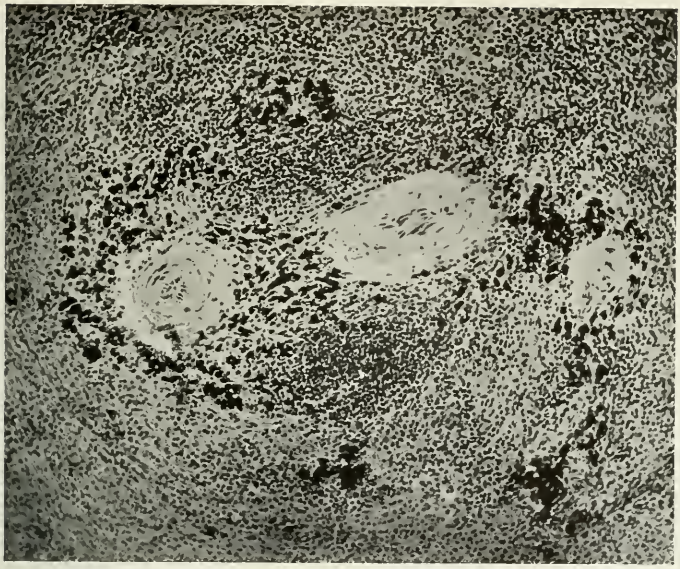


FIG. 18.

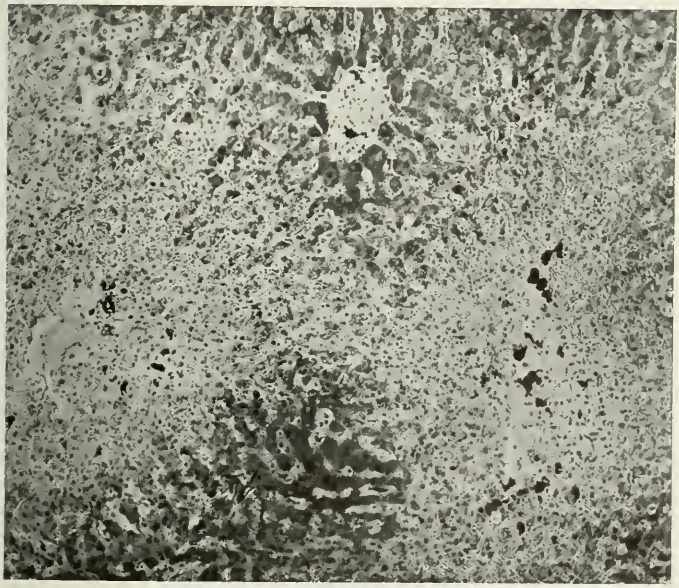


FIG. 19.

THE ETIOLOGY OF ACUTE DYSENTERY IN THE UNITED STATES.[1]

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(From the Pathological Laboratory of the University of Pennsylvania.)

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The etiology of acute dysentery has been worked out in Japan by Shiga,[2] in the Philippines by Flexner[3] and by Strong,[4] and in Germany by Kruse,[5] but before the past summer no systematic attempt had been made to discover the cause of acute dysentery in this country. Our problem has been (1) to determine by comparative study whether the organisms described by these various observers are not really of the same species, though possessed, it may be, of individual differences and peculiarities, such as may readily exist within

[1] This investigation was conducted under a grant from the Rockefeller Institute of Medical Research.

[2] *Centralbl. f. Bakteriologie*, 1898, xxiii, p. 599; xxiv, pp. 817, 870 and 913.

[3] *Bulletin of the Johns Hopkins Hospital*, 1900, xi, pp. 39; 231. *Philadelphia Medical Journal*, 1900, vi, p. 414.

[4] *Jour. Amer. Med. Assoc.*, 1900, xxxv, p. 498, and Report of the Surgeon General of the Army, Washington, 1900.

[5] *Deutsche med. Wochenschrift*, 1900, p. 637.

the limits of a single species, and (2) to discover the cause of acute dysentery in this country, and if possible to identify it with the organisms of the observers mentioned. The problem is, to say the least, rather large. If we have succeeded, we have gone far towards proving that acute dysentery is the same the world over, and is due to a specific microorganism, *Bacillus dysenteriæ* Shiga. How far we have succeeded must be left to the judgment of those who read the following report of our work. In so far as we have attained that success, the credit must be given mostly to Dr. Flexner, who suggested to us this line of investigation, was enabled to put at our disposal the means to carry it on, and gave us the results of his experience in a personal and almost daily supervision of the work.

In the following report we shall give a brief description of the technique, materials and results of our work.

TECHNIQUE.

The materials at our disposal consisted of the stools of persons supposed to have dysentery and the intestines of several fatal cases of the disease. The stools were examined in the majority of cases, and were collected either in a bed-pan, which was previously cleansed and partially sterilized by boiling water, or upon sterile gauze. In some cases a sterile spoon was introduced into the rectum and the cultures made from the material thus obtained. The stool was first examined microscopically for amœbæ, blood, bacteria, pus cells, etc. Three bouillon suspensions were then made, using three to ten platinum loopfuls of the dejecta for each tube of bouillon, according to the number of bacteria judged to be present as determined by microscopic examination. A series of four agar plates was prepared from each of these suspensions, so that in all cases at least twelve plates were made, and often this number was increased by making additional suspensions and plates. In inoculating the agar tubes, six loopfuls of the suspension were carried to the first tube, and so on to the fourth tube, always using six loops. With this method some of the plates would always have the right seeding, though whether the best plate would be the first or the last depended upon the number of bacteria originally present in the stool. When cultures were made at autopsies, the part of the intestine most affected, usually the sigmoid flexure, was examined, taking all the necessary precautions, such as burning the surface of the gut, to avoid

contaminations. The suspensions were made, just as described above, by inoculating the tubes of bouillon with the material in the lumen of the intestine, and also with scrapings of the diseased mucosa.

The plates were incubated for 24 hours, and then all the colonies were marked, this step being described more in detail below (p. 195). The plates then were again incubated for another day, and from the new colonies all those which appeared to have the characteristics of B. dysenteriæ were inoculated by a simple stab in tubes of glucose agar. In order to select the right colonies, a very careful examination with both the naked eye and the microscope is necessary, and even then it is hard to exclude positively B. coli. Inasmuch, however, as the colon bacillus produces gas in glucose agar, while the dysentery bacillus does not, this step in the technique finally excludes all cultures of the colon bacillus.

As soon as inoculated, the glucose agar tubes were placed in the incubator, and remained there for 24 hours, when those that had not produced gas were subjected to further examination. At this stage we always looked for motility, but the morphology cannot be studied satisfactorily at this period on account of the early involution forms produced in glucose agar. Plain agar slants were now made from the glucose agar, and on the former the involution forms quickly disappear, so that at the end of 12 to 24 hours these cultures permit the study of the morphology and can also be used to make fresh suspensions of the suspected organism in order to test agglutinating properties with dysenteric sera. The technique of this test is exactly like that of the Gruber-Widal reaction in typhoid, so that it need not be here described. If the organism, as seen on the agar slant, presented the correct morphology, it was at once inoculated in all the common culture media.

Before the organism under consideration can be considered to be B. dysenteriæ, it must have fulfilled the following requirements:

a. It must give the proper cultural characteristics, as shown by standard cultures of Shiga, Flexner, Kruse, etc.

b. It must possess the right morphology, as shown by the same.

c. It must give a positive agglutinative reaction with some of the known dysenteric sera.

It is obvious that in many cases it will be impossible to obtain the organism in pure cultures on the plates, owing to the large numbers of bacteria present in the intestine under all conditions.

RECORDS OF CASES STUDIED.

1. *Philadelphia Cases.*—These may be said to have been unfavorable cases for the purposes of our study. The stools and autopsies were obtained from various hospitals in the city, chiefly the Philadelphia Hospital, and there was in nearly every instance an unavoidable delay in the transportation of the specimen to the laboratory. It is our belief, judging from the results obtained in these cases, that Bacillus dysenteriæ is present in overwhelming numbers in the intestines of patients suffering with dysentery, and that the colon bacillus is also present but in very small numbers; that the dysentery bacillus does not find the conditions in the stool after evacuation so favorable to its growth as does the colon bacillus, and that therefore in the stool the latter soon overwhelms the former, and if Bacillus dysenteriæ is to be found, the fresh stool should be examined immediately. Scrapings from the intestinal mucosa are more favorable than the stool even when freshly collected.

Case 1. Kelly. The bacteriological examination in this case was made from the intestine after autopsy, the patient having died with the clinical diagnosis of acute dysentery. Unfortunately the patient had been dead for more than twelve hours before the autopsy could be performed.

The intestinal mucosa was greatly injected and studded with minute ulcers, together with some of large size (1 ctm. in diameter). The lumen of the intestine contained bloody mucus and flakes of grayish membrane were adherent in places to the walls. The intestine was opened with the usual precautions, and bouillon suspensions were made both from the bloody mucus in the intestine, and also from scrapings of the mucosa. These were carried to the laboratory and agar plates were immediately made from them.

Out of 18 glucose agar tubes inoculated from the plates, 14 consisted of B. coli, 3 of unknown organisms, and 1 was a pure culture of a bacillus, giving the typical cultural characteristics, to be described later, of the bacillus of dysentery, and also positive agglutination reactions with blood from cases of dysentery (vide infra).

This case is one of the examples of the rapid growth of B. coli and of the equally rapid disappearance of B. dysenteriæ, when conditions

cease to be favorable to its growth. Fifteen hours elapsed between the death of the patient and the time of making the plates.

Case 2. Smith. In this case the stool was the subject of examination. It was very small, liquid, and bloody, and also contained much mucus. Fæcal odor was entirely absent. Microscopic examination showed a field of almost pure blood. Bacteria were not numerous and were entirely bacilli of colon-like morphology, many of which were motile; pus cells were also found. The stool was absolutely fresh when the bacteriological examination was begun. Ten glucose agar tubes were made from the plates with the following results: No colon bacilli were found; two tubes contained organisms that were easily excluded, and eight were pure cultures of the bacillus believed to be B. dysenteriæ.

Case 3. Davis. Specimen obtained at autopsy. The appearance of the large intestine resembled that of the other Philadelphia cases. The lower portion of the colon was the part chiefly affected. A false membrane was present, and the mucosa showed areas of necrosis. The intestine contained little fæcal material, but the liquid present was not particularly bloody. The walls of the intestine were greatly swollen. Plate cultures were made from the scrapings of the mucosa, and all colonies appearing after 24 hours were marked. The later colonies which seemed to resemble those of the dysentery bacillus, were transplanted to glucose agar. Twenty-four tubes were inoculated, and of these 18 did not produce gas. On examination of the latter, 4 cultures gave the serum reaction with several positive bloods, and also characteristic growths on the various culture media, corresponding with B. dysenteriæ of the authors mentioned in every particular. The other cultures were negative. Unfortunately we were unable to test the organisms with the patient's own blood, as we tried to do in all our cases, because nothing was sent but the specimen of the colon.

Case 4. Vincent. The specimen that we examined consisted of a portion of the large intestine obtained at autopsy, the patient having died with the clinical diagnosis of acute dysentery. The greater part of the large intestine was found to be affected, the lesions extending from the splenic flexure to the rectum. The walls of the intestine were thickened the mucosa dotted with small ulcers, and also exhibiting in many places small scars, probably the result of a previous attack. The ulcers were of a greenish-brown color, and a pseudo-membrane was everywhere present. Agar plates were made from scrapings of the

mucosa in the usual way, and promising colonies were transplanted to glucose agar. Out of 41 tubes, all but 8 proved to be B. coli or some other gas-producing organism, and of these eight tubes all were finally excluded except one tube which contained a pure culture of B. dysenteriæ, giving a positive agglutination reaction with Shiga's anti-dysenteric serum,[4] and corresponding entirely in morphology and manner of growth with the cultures of Shiga, Flexner, etc.

Case 5. Examined at autopsy. The whole of the large intestine was affected, and the morbid process extended several centimetres even into the small intestine, but the chief seat of the lesions was in the sigmoid flexure. The mucosa was ulcerated, swollen, congested, and covered with a fine granular exudate. Cultures were taken in the usual manner by scraping the mucosa, including that of the appendix, and from the mesenteric glands. All the organisms isolated from the appendix and glands proved to be either B. coli or B. proteus, but from the cultures made from the scrapings of the mucosa, 18 out of 24 glucose agar tubes proved to be pure cultures of B. dysenteriæ, giving the agglutination reaction with Shiga's anti-dysenteric serum, etc., and agreeing with the standard cultures in all particulars.

2. *Lancaster Cases.*—These were from the Lancaster County Almshouse and Insane Asylum. We are greatly indebted to Dr. Samuel Miller, the Medical Director of that institution, for notifying us of the epidemic, and for that continual courtesy which enabled us to pursue the work to a successful termination. The epidemic had been very severe, having led to a number of deaths; but at the time of our studies it was on the decline, and only three cases were obtainable. Of these, two had been acute but they had greatly improved under treatment, while the third (Benson) began with symptoms of acute dysentery, which, however, soon subsided, and the attack seemed to have been aborted. These cases, therefore, were not so favorable as many of the others which we studied.

Case 6. Benson. The specimen examined was a very small, grayish-brown, liquid stool, which seemed to the naked eye to contain pus, but no blood and very little mucus. Its odor was strong and rather characteristic, but not at all fæcal. Microscopic examination showed many pus cells, some blood corpuscles, and many bacteria—mostly

[4] A small vial of this serum was sent to Dr. Flexner by Dr. Shiga in October, 1900.

bacilli of colon-like morphology, together with some of a larger size. Many were motile.

The attempt to isolate B. dysenteriæ by means of the regular technique resulted as follows: Out of ten glucose agar cultures made from plate colonies, three proved to be of gas-forming organisms, three were of other organisms, probably of the proteus group, and four were pure cultures of B. dysenteriæ, giving the positive agglutination reaction and cultural characteristics described below.

Case 7. Franklin. The very small liquid stool consisted mostly of pus with a little bloody mucus. Microscopic examination showed blood and large quantities of pus cells. Bacteria were not very numerous, and were nearly all bacilli of variable morphology, many being motile. The stool was immediately plated, and of fifteen glucose agar tubes inoculated with colonies from the plates, thirteen contained gas-producing organisms, and two were pure cultures of an organism giving all the cultural characteristics of B. dysenteriæ, and also good agglutination reactions with the patient's own blood serum and with several other dysenteric sera. It may be mentioned here that Franklin was an insane patient.

Case 8. Hoffman. The stool was large in quantity, liquid and almost entirely fæcal; no blood or mucus was found. Microscopic examination failed to show blood, but demonstrated the presence of large numbers of bacteria, most of them of colon-like morphology and actively motile. The stool was at once plated, and of the twenty glucose agar tubes inoculated from the colonies, fourteen were cultures of a gas-producing bacillus, four were of other organisms not identified, and two were pure cultures of B. dysenteriæ.

These last two cases were convalescent, and Hoffman was practically well of his dysentery. The bacteriological examination of the stools showed that the dysentery bacillus had begun to disappear, and that the colon bacillus was resuming its place as the dominant microorganism of the intestinal tract. The conditions here were in marked contrast to those in the New Haven cases, which were very acute, and gave in the glucose agar tubes nearly pure cultures of Bacillus dysenteriæ.

3. *New Haven Cases.*—These cases were obtained at the Springside Home, New Haven, Conn. The epidemic was very severe, fifty or more cases occurring within three weeks among the 350 in-

mates, all being of the very acute form, with fatalities. The patients were stricken suddenly, and were very ill for a few days, their evacuations being extremely bloody and mucoid, containing little or no fæcal material, and being odorless during the height of the disease. Thanks to Dr. Gompertz, Visiting Physician to the Home, and to the hospital assistants, every opportunity was afforded for thorough investigation.

Case 9. Alyvard. The bacteriological examination was made from a fresh stool, which was large in quantity, liquid, very bloody and mucoid. Microscopic examination showed numerous pus cells, and bacteria which were almost all bacilli. From favorable colonies on the agar plates, seventeen glucose agar tubes were inoculated, out of which twelve tubes afterwards proved to be cultures of B. dysenteriæ.

Case 10. Tedymus. Bacteriological examination was made at the autopsy. The colon from the splenic flexure to the rectum was the seat of ulceration, intense hyperæmia and pseudo-membranous formation. Cultures were made from scrapings of the mucosa and also from the contents of the intestine in several localities, with the result that out of twenty-seven glucose agar tubes, eighteen contained cultures of B. dysenteriæ, the majority being obtained from the scrapings of the mucosa rather than from the intestinal contents.

Case 11. Seward. The stool from which the cultures were taken, was liquid, very bloody, mucoid, and devoid of odor, with no fæcal material present. It was obtained fresh and plated in the usual manner. Out of twenty-four glucose agar tubes, twenty-one proved to be pure cultures of B. dysenteriæ. The patient subsequently died and an autopsy was performed. On opening the intestine, the lower two-thirds of the large gut was thickened, ulcerated and closely dotted with small hæmorrhagic areas. No considerable pseudo-membrane was found. Plate cultures were made from scrapings of the mucous coat, and ten glucose agar tubes were finally inoculated from colonies, all of which contained B. dysenteriæ. These results obtained from the same case, which was particularly favorable for our study, both from the stool during life and from the intestine at autopsy, indicate that the latter method when applicable offers the best chances of success.

Case 12. Cook. The specimen was a stool, typical of the acute malady. The patient was in a comatose state from which she could not be aroused. The movements of the bowels were continuous and contained no fæcal matter, consisting of blood and mucus. Microscopic

examination showed large numbers of pus cells and red corpuscles, and many bacteria. In this case cocci were rather numerous. Owing to the state of the patient, the evacuations had to be collected in a bed pan under unfavorable conditions, which may account for the small number of successful cultures from a case apparently so favorable. Suspensions were made from the material thus collected, which was by no means fresh, and the regular technique was followed, but out of ten glucose agar tubes but two finally proved to contain B. dysenteriæ.

Case 13. Kenney. Stool liquid and bloody, with a little fæcal matter. Microscopic examination showed quantities of pus cells, and many bacteria, which were mostly bacilli. Out of thirteen glucose agar transplantations from colonies, seven did not produce gas, and further examination showed these to contain a non-motile bacillus which responded to all the tests for B. dysenteriæ.

Case 14. Higgins. From a typical liquid and bloody stool, suspensions and plates were made in the usual manner, and eight out of ten glucose agar inoculations did not produce gas. One of these cultures proved to be of a very motile bacillus; the other seven were of the same organism isolated and described in the foregoing cases, giving the serum reaction with the patient's blood as well as with other positive sera, as shown in the tables on pages 201-2.

Case 15. Perkins. This was another case of dysentery in the insane. The stool was extremely bloody, and did not differ from the others in this series. Twelve out of fourteen glucose agar cultures were non-gaseous, and of these four were excluded, since they contained a short, thick, motile bacillus, not corresponding also in other respects to the bacillus of dysentery. The other eight cultures were of non-motile organisms, giving the agglutination reaction with other positive bloods, but not with the patient's serum. The cultures of bacilli from Shiga, Kruse, Strong, and Flexner also failed to react with the patient's serum. The patient died after a few days' illness, and it is possible that he was not ill long enough for the agglutinating properties to develop in his blood. We wish to lay especial stress upon the fact that the organisms isolated did give positive reaction with other known dysenteric sera.

Case 16. Parmlee, also an insane patient. The stool was streaked with blood, but also contained considerable fæcal matter. Nine out of sixteen glucose agar transplantations, contained pure cultures of B. dysenteriæ. These gave the agglutination reaction with the patient's own blood serum, as well as with the other positive sera in our posses-

sion. The patient's serum had been previously tested with Shiga, Flexner, Kruse, and Strong cultures, and gave positive reactions with all these, thus proving that it was a positive serum.

The results obtained in these three cases of dysentery in the insane were therefore identical with the results furnished by the other cases of acute dysentery that we had the opportunity of studying.

Case 17. Dobell. The examinations were of a stool that appeared in every way typical. Out of twenty-four glucose agar tubes inoculated with apparently favorable colonies, seven contained a non-motile organism with a variable morphology, and failed to give the agglutination reaction. The other fifteen gave this reaction and corresponded in morphology and cultural characteristics with the standard cultures.

Case 18. Kittler. This stool was not very favorable as it contained considerable fæcal material and was only streaked with blood in places. Four out of twelve glucose agar transplantations gave the agglutinating reaction, and agreed in other respects with the cultures of Shiga, Flexner, etc.

Case 19. Prescott. The stool was large, liquid, and contained finely divided fæcal particles, together with some blood. Out of eighteen glucose agar tubes five contained the same organism isolated from all the foregoing cases.

Case 20. Cunningham. The stool contained a few pus cells, and was semi-solid in consistence. It was fæcal in character and had a bad odor, as did the other stools containing fæces. We were successful in isolating B. dysenteriæ in nine out of fifteen glucose agar tubes.

Case 21. Howard. The stools throughout the whole course of this case were large, and, while containing considerable blood, were at no time free from fæcal matter. Out of eight glucose agar inoculations, only two gave the characteristic agglutination reaction and cultural properties shown by the standard cultures. The clinical history of the case and the character of the stools show that this was by no means so acute a case as most of the others at New Haven, and in this respect as well as in the smaller percentage of successful glucose agar tubes it resembles those of the Lancaster epidemic.

Case 22. Wells. Stool semi-solid and fæcal, but streaked with blood and mucus. Three out of eight glucose agar transplantations contained B. dysenteriæ. The epidemic was by this time on the decline, as is shown by the character of the stools of the last two or three patients, and after this, no other typical cases appeared at New Haven.

CULTURAL CHARACTERISTICS.

With the view of determining how close the relationship between the various bacilli described by Shiga, Flexner, Kruse, and Strong, really is, a series of parallel cultures of all of these was made, beginning with agar plates, and afterwards carrying them through all the common culture media. Later the bacilli from our own cases narrated above were also included and made a part of the series.

In the course of this study we observed a few slight differences between the varieties of the several observers. For example, the colonies of one variety might be a trifle darker in color than those of another; or one variety might, at a given age, have slightly larger colonies than another; or one variety might produce a greater amount of acid in litmus milk than another. But these differences were nowhere greater than might be expected of individuals of the same species, and moreover they were by no means constant, but might be noticed on a first trial and be absent on a second or third. We endeavored to rule out the personal equation in the following way: One of us would make a series of cultures or plates, keeping the names hidden, and the other would try to identify them. We varied the experiment by requesting other laboratory workers, not interested in the problem, to distinguish between the various cultures, but such attempts always failed.

We are therefore forced to the conclusion that the cultural characteristics of the various forms studied are essentially alike, for, however they might vary when we knew them by name, these variations were so inconstant that it was impossible to distinguish one culture from the other when the names were hidden. In fact these slight variations must be considered as one of the peculiarities of the organism, just as a rather wide variation in morphology is also characteristic. Since we have been unable to discover any real or constant differences between the varieties, it would be a vain and tiresome repetition to give the cultural characteristics of them all seriatim, as we had at first intended, and we shall therefore be contented with giving a careful description of the characteristics as we observed them, with the understanding that this description fits any one of the

varieties studied, including those which we isolated the past summer.

Surface Colonies.—The appearance of the colonies of course changes gradually, but it is convenient to describe several stages.

Twelve hour stage.—The colonies are circular in outline, and about one millimetre in diameter; to the naked eye they are whitish, but very translucent, and resemble ground glass in color. The margin of the slightly raised colony is perfectly smooth and regular. The colonies are smooth in texture, shining, and resemble the icing on a cake. Under the microscope, they are finely granular throughout, and so translucent that it is often difficult to focus them. The central part by transmitted light is pale yellow, gradually fading to a lighter shade towards the margin, while the outer third is absolutely colorless, thus giving the impression of two zones, which fade one into the other so gradually that there is absolutely no line of demarcation. Even under the microscope, the margin is almost mathematically regular and accurate.

Twenty-four hour stage.—The appearance now is also quite characteristic. The colonies have reached a size of from two to four millimetres, and are more nearly creamy in color and general appearance. Their texture is not so smooth, nor are they so shiny and refractive; they are also more elevated above the surface, though this is not a marked feature at any stage. They are still somewhat translucent, and their outline is circular and regular as before. Under the microscope, however, the margin is by no means so regular as it was at twelve hours, but presents a slightly ragged appearance, and the most striking change of all is, that there are three zones instead of two. In the centre a circular nucleus is seen of a light yellowish-brown color, very distinctly darker than the surrounding area. The nucleus is granular like the rest of the colony, but the granules are a little coarser, while the granulation of the rest of the colony is as fine as it was in the twelve hour stage. Around the nucleus is a pale greenish-yellow area, deepest in color towards the centre, and fading gradually away towards the periphery until it merges into the last or outer zone, which is absolutely colorless like that of the twelve hour stage, but has become more limited in extent.

Thirty-six hour stage.—The macroscopic appearance is very similar to that of the twenty-four hour stage, except that the colony is now larger, but the microscopic characteristics are much changed. The outline of the colony varies considerably in different colonies. Often it is still fairly regular, but in many instances it is decidedly ragged; always, however, it is approximately circular. The most apparent change is in the nucleus. This has become irregular and ragged in outline, of a deeper brown color, and is surrounded by a number of small lumps of irregular shape, size and arrangement, which have apparently been broken off from the nucleus. The color and granulation of these globules is the same as of the nucleus, in which the granules have, by this time, become so dense and thickly packed, that individual ones are no longer readily visible. It is also very noticeable that the granulations throughout the remainder of the colony are much coarser than before, and the area of the clear colorless outer zone is much diminished. The general color of the colony is a deeper yellow than before, and the translucency is greatly diminished.

Forty-eight hour stage.—The colonies now average from five to six millimetres in size, although there is much variation in this, many being smaller. They are quite white, often rather rough in texture, are no longer translucent, but are still circular in outline. The changes seen under the microscope are the same in character as those noticed before, but they are much more pronounced. The changes in shape may be summed up by saying that the tendency is for the granulations to become much coarser, so that at this stage, the colony is lumpy rather than granular. The nucleus is no longer circular in outline, but is usually oval, often lobulated, and of a deep brown color. The entire colony, with the exception of a small area around the periphery, is closely packed with irregularly shaped globules of all sizes, but the larger ones are mostly grouped around the nucleus and these are of the same color and structure as the nucleus, but of a lighter shade. Toward the margin of the colony the globules become more scattered, and less closely packed, until, as already noted, there is a narrow band around the periphery where there are hardly

any globules or lumps, but which is filled with very coarse granulations. Even in the old colonies, the outer rim is nearly colorless, a gradual transition taking place between the deep brown of the centre and the gradually fading brownish-yellow of the outer portion. Often colonies are found in which the nucleus is entirely broken up, and the only difference between the central portion and the remainder is that the lumps are rather larger.

Beyond this stage, the changes are neither particularly characteristic nor interesting, and consist merely of a further disintegration of the structure of the colony, which reaches its point of highest development in the twenty-four hour stage.

The *deep colonies* are of either a lenticular or irregularly spherical shape. To the naked eye, they are dirty yellow in color, but are yellowish-green under the microscope by transmitted light. They are smaller than the surface colonies, and are finely but thickly granulated. Their margins are at first quite smooth, but as they grow older, they become lined with protrusions and excrescences, and finally become lobulated to such an extent as to lose their original shape. Coincidentally with this change they become deeper in color, until finally they are of a rather deep brown. They reach their typical appearance at about the twenty-four hour stage, and the lobulation and deepened color are quite characteristic after forty-eight hours' growth.

It is seen from this description that the colonies are very similar to those of the colon and typhoid bacilli. In our work of isolating Bacillus dysenteriæ from the stools, we were never troubled by having to distinguish between that organism and the typhoid bacillus, because of the ease of distinguishing clinically between dysentery and typhoid fever, but B. coli was a continual thorn in the flesh. Not only is it present in practically all stools except those of the very acute dysenteries, but when it is plated out, it is almost impossible to distinguish it from B. dysenteriæ. Of course, all cultures of the colon bacillus unintentionally made from plate colonies are easily excluded in the glucose agar stage of the technique, but the real difficulty in all but the very acute cases, is to succeed in getting any cul-

tures of B. dysenteriæ at all, since the colon bacillus is present in vastly greater numbers, and, without any sure method of distinguishing the two species, most of the cultures would according to the law of chances prove to be B. coli. Therefore we have been continually on the lookout for a ready method of distinguishing the two species, and we think we have been successful to a considerable extent.

The colonies of B. dysenteriæ do not grow so luxuriantly as those of B. coli, and are always paler, smaller, and less white, and can usually be distinguished in this way. After experimenting along several lines, we have added to our regular technique a modification that we believe to be of great assistance in separating the dysentery bacillus from the colon bacillus. This is based upon the more rapid growth of the latter. The plates are made in the usual way, and are set in the incubator for twenty-four hours, when they are taken out and every surface colony on the plate is marked by scratching on the glass directly over it with a blue wax pencil. The plates are now put in the incubator again for from 12 to 24 hours, and when they are examined at the end of this period, it will be found that many new colonies have come out. It has been our experience that, although among the colonies that have thus developed after the first twenty-four hours, there will be a few colonies of B. coli, by far the greater proportion will be of the dysentery bacillus, and that this method therefore renders very valuable assistance in isolating the latter.

Ordinary Culture Media.—Here, as in the case of the plates, the cultures from the different sources mentioned presented essentially similar characters. Shiga's and Kruse's bacilli sometimes produced in litmus milk a little more acid at first than either Flexner's or Strong's bacillus, but after the subsequent change to the alkaline reaction one culture could not be distinguished from another. The organisms isolated in this country also coincide in all particulars with those of the observers mentioned.

Plain agar.—At *twelve hours* a thin but well-marked, dull grayish-white, translucent growth is observed all along the line of inoculation, having a perfectly regular margin.

At *twenty-four hours* there is an increased growth, with a uniform lateral spreading, but otherwise not much change.

After *thirty-six hours* the edges of the growth become rather uneven, and the lower part of the streak is somewhat wider than the upper portion. It is raised somewhat above the surface, more especially in the centre of the streak, and the translucency is now about gone, the color being more nearly creamy white.

At *forty-eight hours* the margin of the streak has become crenated, especially at the lower portion, while the whole of the growth is denser and more elevated.

After *seventy-two hours* often a slight depression may be noticed in the centre of the streak, and the color is a decided cream white.

From now on, the principle change of interest takes place along the margin of the growth, and this may be said to be characteristic. Especially if the agar is still fairly moist, the margin tends to become feathery, slender processes budding out from the sides, and branching dichotomously. Inasmuch as this process is more marked towards the bottom of the slant, there is a gradual tapering off towards the top, with the result that the growth now reminds one strongly of a northern fir.

Gelatin.—After *twelve hours* of incubation, there is a fine film-like growth along the path of the needle. The growth on gelatin is neither so rapid nor so profuse as on agar.

At *twenty-four hours* there is no perceptible change, except a very slight increase in the amount of the growth, and even this is often dubious.

At *thirty-six hours*, however, the growth is more clearly visible, and if examined closely, the individual colonies can be made out. There also now begins to be some little growth on the surface, immediately encircling the entrance of the needle. There are no further changes. There is little or no further growth, absolutely no tendency to spread out over the surface of the gelatin, and at no period is there liquefaction or clouding of the medium.

Potato.—At *twelve hours* there is a slight growth visible, which is rather translucent, so that the tube must be held in a proper light to

permit the growth to be seen readily, the margin is irregular, and at first there is no coloration.

At *twenty-four hours* the growth is fairly profuse, of a faint yellow color, with a shiny surface, spreading rather freely, with irregular edges.

At *thirty-six hours* the growth still continues to spread beyond the line of inoculation, and is raised above the surface of the medium.

At *forty-eight hours* the surface of the growth has become roughened, with a yellow color centrally that fades to a gray towards the periphery. The potato is usually somewhat discolored around the growth.

At *seventy-two hours* the growth seems to cease, and becomes depressed in the middle portion. Beyond this stage there seems to be very little change, until the time when the potato becomes dry and shrivelled.

Blood serum.—The growth is about as rapid and profuse as upon agar. At *twelve hours* it is very easily discernible, though in color it can hardly be distinguished from the medium, and is shiny and translucent. The margin is regular.

At *twenty-four hours* the streak is larger in extent, and tends to sink below the surface of the medium, probably on account of some slight liquefaction, so that it gives the impression of being inlaid. The margin has become slightly corrugated.

After *thirty-six hours* there is very little change. The streak may increase slightly in size, but there is nothing further characteristic.

Glucose agar.—At no period is gas produced.

At *twelve hours* there is a distinct granulated growth along the path of the needle, of a dirty grayish-white color.

At *twenty-four hours* the quantity is increased, and there is a small irregular circle of growth surrounding the point of entrance of the needle on the surface. This tendency toward surface growth seems to be strictly limited, for it only spreads a few millimetres, and then no further change is observed. After some days, the medium becomes slightly and uniformly clouded.

Bouillon.—A cloud is produced in several hours, which becomes

gradually heavier, until at the end of twenty-four hours, it is very dense and a sediment fine and silt-like in character has begun to fall to the bottom of the tube. This sedimentation continues for several days while the supernatant liquid becomes gradually clearer and clearer, until finally the sediment is several millimetres in depth, and the supernatant fluid is nearly as clear as before inoculation. At no time is there a pellicle formed.

Bouillon containing one per cent of saccharose, lactose, and glucose was prepared and inoculated. The growth was similar in character to that of the plain bouillon, and no gas was produced at any stage, with any of the sugars.

Litmus milk.—After twelve hours, there is a varying amount of acidity, but at no period is there any tendency toward coagulation.

At thirty-six hours the milk is still acid, but the reddish shade is not so pronounced, and at forty-eight hours it is very evident that a gradual change back to the original color of the medium has set in. For a varying period of time thereafter, the milk remains alkaline. This state of affairs usually lasts several weeks, when there may be a second production of acid, but one very much less noticeable than the first stage of acid production. If the culture is kept for several months, it may become perfectly white. This condition, brought about by the abstraction of oxygen from the litmus, may be readily overcome by shaking the tube and thus bringing the liquid into a more intimate association with the oxygen of the air, when the litmus assumes a bluish tint.

MORPHOLOGY.

The bacillus is a slender rod with rounded ends, about 1 to 3 μ long. It has no tendency to form groups, and is usually found singly, but sometimes in pairs. It stains readily with the ordinary aniline dyes, is not stained by Gram's stain, possesses flagella, and a capsule is sometimes present. Involution forms develop on glucose agar in a very short time, often in twelve hours. Under these conditions the bacilli are very much larger in every way and of strikingly irregular form.

Flagella.—After many unsuccessful attempts we succeeded in demonstrating the presence of flagella. They are numerous, entirely surround the body of the bacillus, and are eight to ten times its length. They are very delicate, and are arranged in a wavy manner that recalls the delicate hair-like fibrils of floating seaweed. In other specimens in which the flagella were more broken up by the manipulations, they simply form a net work radiating in all directions, without creating the beautiful picture referred to above. To stain the flagella is a very difficult task, inasmuch as no directions can be given that will ensure success without a good many preliminary trials. Even when we felt that we had the method perfected, we did not succeed to our satisfaction oftener than once out of ten or fifteen trials. The following method is the one with which we were most successful, and is a modification of van Ermengem's method. On an absolutely clean cover slip place a drop of sterile distilled water, and inoculate this with bacteria obtained from a plain agar culture not more than twelve hours old, being careful not to make too heavy a suspension. Cover the slip carefully to prevent dust from falling on it, and allow it to stand for 15 to 20 minutes. This is to allow the bacteria to become scattered over the surface, without the necessity of spreading them with the platinum needles, which breaks up the flagella. We have never obtained a specimen with the flagella unbroken that has been spread with a needle. Dry in the air, without heating at all, and then pass the slip once through the flame for fixation. Now cover the slip with van Ermengem's solution no. 1, and allow it to remain for two or three hours, without heating it, adding enough of the solution from time to time to prevent its drying on the slip. The mordant is to be washed off very gently with distilled water, until it is all removed, and without drying the slip, the second solution, silver nitrate 0.6% is dropped on. This is to remain for five minutes, when it is poured off, and the slip is placed in van Ermengem's solution no. 3. This is allowed to remain until the color is a light brown (not dark), when it is poured off and the film is washed again in distilled water, and cleared in 1 to 1000 acetic acid, and again washed in water, dried and mounted. It will be seen from this account, that

our principle modifications consisted in not heating the mordant, but in allowing it to remain on for a much longer time in the cold, in not using any alcohol after the mordant, and in using a somewhat stronger silver nitrate solution than van Ermengem gives in his formula.

Motility.—We can only say with regard to this point, that in none of our cultures was motility observed, and this applies to the organisms of Shiga, Flexner, Strong, Kruse, and those that we isolated. After having demonstrated the presence of flagella, it is at any rate possible, and even probable, that at some period of their existence, or under favorable conditions as yet undetermined, the bacilli are motile; but we have looked for motility at almost all ages, and under varying conditions, and have never succeeded in finding it.

Pathogenic Properties.—The pathogenicity of the bacillus has up to this time been little studied by us by experimentation upon animals. Guinea-pigs succumb to intraperitoneal inoculation in less than twenty-four hours, the lesions consisting in those of a sero-fibrinous peritonitis. Bacilli are abundant in the exudate, and may also be cultivated from the heart's blood.

AGGLUTINATION REACTIONS.

Much time was devoted to a study of the agglutination reactions of the bacilli isolated by us and, at the same time, of those cultures with which we first worked, namely, Shiga's, Flexner's, Kruse's and Strong's. The blood for the tests came from a wide series of cases, including the majority of those from which stools were studied, and some in which stools or autopsies were not obtainable. In addition to the human blood we were supplied with the sample of anti-dysenteric serum sent by Dr. Shiga to Dr. Flexner. Some interesting results were also obtained with several specimens of blood sent by Dr. Ross from Morven, North Carolina, where a short time previously acute dysentery had prevailed.

In all cases, control tests of the blood sera were made with suspensions of B. coli and B. typhosus, and normal blood was frequently used as a control for the specific sera. We never obtained an agglutination in any of these control tests, no matter what dilution was used.

The tests consisted (1) of the reactions of the patient's own blood with the cultures of Shiga, Flexner, Strong, Kruse; (2) of the reactions of the bacilli isolated by us with the patient's own blood and with other sera that had been previously tested and proved to possess agglutinating properties toward B. dysenteriæ; and (3) the reactions towards Shiga's anti-dysenteric serum.

In order to avoid repetition and the use of unnecessary space only two tables will be given. They represent the results of tests made with the sera obtained from cases of dysentery and with the anti-serum of Shiga. The bacilli and the blood are designated by the name of the patient from whom they were obtained. The first column gives the source of the serum, the second of the culture, the third the dilution employed, and the fourth the result. The final result may represent a reading as late as several hours after mixture of the culture and diluted serum.

TABLE I.

AGGLUTINATION REACTIONS WITH BLOOD OF PATIENTS.

Blood.	Suspension.	Dilution.	Result.	
			1 hr.	Final.
Cook	Shiga	1–30	+	+
"	Strong	"	+	+
"	Flexner G	"	+	+
"	" H	"	+	+
"	Kruse	"	+	+
"	Vincent	"	+	+
"	Davis	"	+	+
"	Vincent	1–50	+	+
"	Strong	"	+	+
"	Flexner G	"	+	+
"	" H	"	+	+
"	Cook	1–16	+	+
McShara	"	1–500	+	+
Kinny	"	1–200	+	+
Alyvard	"	1–20	+	+

The uniformly positive results in the tables given serve as examples of the numerous additional tests made.

Certain variations in the appearance of the clumping were observed. The forms taken by the masses of bacilli may be divided into two groups: in the one, the bacteria were tangled in tight bunches, as is

commonly seen in the Gruber-Widal reaction: in the other, the bacteria were united end to end and thus formed long threads which were interlaced so as to give rise to a loose skein. This latter appearance has been noted both by Kruse and by Flexner, but it seems to be the exception.

TABLE II.

Agglutination Reactions with Shiga's Anti-Dysenteric Serum (designated A. D. S. in the Table).

	Suspension.	Dilution.	Result. 1 hr.	Final.
A. D. S	Flexner G	1–200	?	+
"	" H	"	?	+
"	Shiga	"	+	+
"	Strong	"	+	+
"	Kruse	"	+	+
"	Flexner H	1–300	—	?
"	" G	"	—	?
"	Shiga	"	+	+
"	Strong	"	+	+
"	Shiga	1–500	+	+
"	Kruse	1–300	+	—
"	Flexner	1–500	—	—
"	B. typh. as control	1–25	—	—
"	Davis	1–200	+	+
"	Vincent	1–100	+	+
"	Tedymus	1–500	—	+
"	Strong	1–500	—	+
"	Sykes	1–50	+	+
"	Alyvard	1–100	+	+
"	Seward	1–100	+	+
"	Cook	1–100	+	+
"	Kelly	1–35	+	+
"	Smith	1–20	+	+

The agglutinating properties do not appear in the blood immediately upon the appearance of clinical symptoms. This is shown by the fact that we have had some patients suffering from undoubted dysentery, from whose stools we secured B. dysenteriæ, and yet their blood serum was negative or nearly so.

The reaction is, moreover, capable of disappearing from the blood in a rather sudden manner. The McShara case of the New Haven epidemic indicates this. When we reached New Haven the patient was convalescent, but her blood reacted with the bacilli of Shiga, Flexner, etc., and also with the cultures from the Philadelphia cases. The dilutions employed were 1 to 200. Two weeks later, fresh

blood being taken, reaction even in 1 to 10 dilutions could not be obtained with any of the bacilli in our possession.

While the agglutinating properties of the bacilli from different sources are very much the same, still there are differences in degree. Certain strains of bacilli react better with a given serum than do others as is shown by the fact that if high dilutions are used, some forms will always drop out sooner than others. We have never made a test with high dilutions in which all the varieties would be positive to a certain point and then all drop out together. Again, with certain weak sera, while certain of the varieties of bacilli were positive, occasionally we have found one or two that absolutely refused to react. It must also be noted that certain sera are very powerful agglutinators, while others, taken from patients with just as severe a dysentery, which has existed quite as long, are comparatively very weak. In general it may be said that the sera obtained from the New Haven patients were very strong, while those obtained from Lancaster, Philadelphia and Morven, N. C., were in almost all cases comparatively weak.

DISCUSSION OF THE RESULTS.

To sum up the results of this study would be to state that the observations of Shiga made upon the dysenteries in Japan, of Flexner upon the same disease in the Philippine Islands and in Porto Rico, and of Kruse in Germany, can be applied to the acute dysenteries of this country. So far as the results of modern bacteriological study can be trusted, all the criteria have been successfully fulfilled in establishing the bacillus obtained from the wide range of cases here reported to be the cause of the dysentery from which the patients suffered—that is, all the criteria which have been set up as the result of the study of the disease in the places mentioned, for in all one condition has been lacking, namely, the production of the disease by inoculation. Of the pathogenicity of the organism, there is abundant proof; and the bacillus isolated by us is likewise pathogenic for laboratory animals. But in no case, unless the effects of the subcutaneous

injections carried out by Flexner[7] be taken as proof, have specific intestinal lesions been produced in animals inoculated with the organism. In two instances related by Flexner circumscribed lesions consisting of swelling and necrosis of the intestinal mucous membrane in rabbits followed the injections. The character of the lesions, only briefly described, agrees with similar appearances which occasionally result from the inoculation of these animals with virulent cultures of B. typhosus and B. coli. In two instances the symptoms of dysentery have followed ingestion of cultures of B. dysenteriæ by human beings. The first is reported by Flexner[8] and was the result of accidental inoculation: the second by Strong,[9] who fed a culture to a Filipino prisoner. Both men developed characteristic symptoms, and Strong recovered the bacillus from the dejecta of his patient.

The present study also bears upon two other important phases of the problem: the cause of sporadic dysentery, and that of the dysentery of institutions, such as those for the insane. The cases arising in Philadelphia were not a part of an epidemic; they were scattered cases, most of them being among the inmates of the Philadelphia Hospital. But others arose in widely removed portions of the city, and were encountered in the Pennsylvania Hospital. Neither did these cases become centres of infection, for at no time in the summer, while this work was being prosecuted, did a considerable number of them occur.

It is also significant that several of the sporadic cases were "terminal" dysenteries. The patients were chronic invalids, and had suffered long from chronic Bright's disease, the autopsy establishing the existence of the small contracted kidney.

The Lancaster and New Haven outbreaks were typical institutional epidemics. Since the appearance of Kruse's[10] second paper it has become of much interest to decide upon the exact nature of the so-called institutional dysentery. Kruse considers that the cause of

[7] *Univ. of Penna. Medical Bulletin*, 1901, xiv, p. 191.

[8] *Philadelphia Medical Journal*, 1900, vi, p. 414.

[9] Report of the Surgeon General of the Army, Washington, 1900.

[10] *Deutsche med. Wochenschrift*, 1901, p. 370.

this is different from that of the epidemic dysenteries in general, and he has been led to choose the unfortunate term of "pseudo-dysentery" to designate the disease as it appears in asylums for the insane. Our studies, which were mainly upon institutional epidemics, show this position to be untenable. For not only are the cultural properties of the bacillus obtained by us from institutional outbreaks identical with those of bacilli obtained from ordinary epidemics, but they agree with the standard cultures used throughout this investigation, and exhibit similar agglutinating reactions with them to positive blood sera.

The question of motility has been somewhat mooted, in that Shiga, Flexner, and Strong all described some motility, while Kruse has never been able to detect it. Our observations are in this regard in agreement with those of Kruse, although the demonstration of flagella has an important bearing upon the ultimate solution of this question.

CONCLUSIONS.

1. The several standard cultures used in this study are indistinguishable—a conclusion previously reached and stated by Flexner.

2. The acute dysentery of the United States is due to a bacillus indistinguishable from that obtained from the epidemics of dysentery in several other parts of the world.

3. The sporadic and the institutional outbreaks of acute dysentery are caused by the same microorganism, and this organism is identical with that causing epidemic acute dysentery.

4. The cause of acute dysentery, whether sporadic, institutional, or epidemic, is *Bacillus dysenteriæ* Shiga.

EXPERIMENTS ON THE EFFECTS OF INJECTION OF EGG-ALBUMEN AND SOME OTHER PROTEIDS.

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Plates XVI–XX.

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INTRODUCTION.

Although the occurrence of albuminuria after injection of certain proteids, notably egg-albumen, has long been recognized, many details of this phenomenon have received but scant attention. At the time when this research was started, in the spring of 1897, very little was known concerning the ratio of the proteid which is excreted, the time required for excretion, and the factors which influence these; nor were there any very definite data about the fate of that portion which is retained, about the changes occurring in the kidneys, and about certain toxic effects produced. Since then a number of important papers dealing with this subject have made their appearance and have in large part anticipated some of our results. However, the topic is even now far from exhausted; many questions have only been touched upon and others will bear further support. We have, therefore, extended our research along lines not originally contemplated, and the presentation of the experiments in the order in which they were performed would not do justice to the subject. We have accordingly grouped them under various headings.

The tables will take the place of more extended protocols.

TABLE I.—EXP. I-XLIV. ABSOLUTE AMOUNTS OF PROTEID INJECTED, RECOVERED AND RETAINED.

Experiment number.	Other experiments done on same animal.	Animal.		Weight in kilos.	Manner of injection.	Solution used.	Absolute amount of proteid injected.	Absolute amount of proteid recovered.	Absolute amount of proteid retained.	Amount of proteid injected per kilo. of animal.
I	(II, III, IV).	Dog	♀	6.13	Vein.	Egg, watery, 1.148%.	1.148	0.7672	0.3825	0.187
II	(I, III, IV), (4 days since last experiment.)	"	"	"	Hypodermic.	" "		Only non coagulable proteid.		
III	(I, II, IV), (6 days since last exp.)	"	"	"	Vein.	Normal salt, 10 cc, 4 min.				
IV	(I, II, III), (6 days since last exp.)	"	"	"	Hypodermic.	Egg, watery, 4.150%.	2.078	1.402 +	Died before excretion was completed.	0.338
V	None.	"	"	5.9	Vein.	Egg, freed from globulin by dialysis, 4.470%.	2.2484	1.463	0.785	0.381
VI	"	"	"	5.3	"	Egg Syntonin in alk. solution. Proteid = 1.276%. Alkalinity = 0.376% NaOH.	1.914	Anuria to death.		0.361
VII	"	"	"	8.75	"	Alkali alb. from egg. Proteid = 0.243%; Alkal. = 0.110%.	0.243	0.0719 alkali alb. 0.0142 coagulable albumen.		0.028
VIII	(X)	"	♂	6.47	Hypodermic.	Alkali alb. from egg. Proteid = 2.447%. Alkal. = 0.316%.	2.227	None.	2.227	0.344
IX	(XI)	"	♀	10.	Vein.	Egg, watery, 0.055%.	0.0453	0.0817	Negative.	0.0045
X	(3 days after VIII).	"	♂	6.47	Hypodermic.	" " 5.230%.	4.1364	1.3325	2.8039	0.639
XI	(4 days after IX).	"	♀	10.	Vein.	Alk. alb. from egg. Proteid = 3.073%. Alkal. = 0.4995%.	6.146	Trace, both alk. and native.	6.14	0.615
XII	None.	"	♂	5.23	Hypodermic.	Egg-albumen.	0.1218	3 days; 0.0135		0.023
XII A	5 days after last.	"	"	"	"	" "	0.0669	4th day; 0.5861 and albumose.	0.06	0.0116
XIII	None.	"	"	4.8	"	" " (free from albumose.)	1st day; 1.5266 2d " 1.6240 3d " 1.2992 6th to 9th day; 1.4078 Total, 5.8576	Trace of albumose. Little albumen, considerable albumose.		1.220 in 9 days.
XIV	"	"	♀	5.7	Vein.	4.742% albumen + 0.5% Na_2CO_3 (unconverted.)	7.7303	Much albumen (urine solid on heating).	Died before complete excretion.	1.356
XV	"	"	"	2.7	"	Egg in normal salt, 1.955%.	1.7146	Much albumen.	Died before complete excretion.	0.635
XVI	"	"	"	2.7 (fat)	"	Egg, watery, 2.350%	1.892	0.7372	1.1546	0.701
XVII	"	"	"	4.55	"	Undiluted egg albumen, (14.8%.)	12.769			2.806
XVIII	"	"	"	4.5	"	Egg, watery, 2.849%.	2.840	1.5826	1.2574	0.631
XIX	"	"	"	4.1	"	" " "	2.849	1.5646 +	Died before complete excretion.	0.702
XX	"	"	"	7.7	"	" normal salt, 0.655%.	0.0721	0.2823 in 8 days, and still excreting.	More excreted.	0.009
XXI	"	"	"	4.8	"	" " " "	0.0629	0.1771 in 5 days.	" "	0.013
XXI A	In 6 days, 0.2 Gm. Morphine.	"	"	"	Hypodermic.					
XXII	None.	"	"	9.5	Vein.	Egg, watery, 1.855%	1.855	[1.4080 in 2½ hours.]		0.195

TABLE I.—EXP. I-XLIV. ABSOLUTE AMOUNTS OF PROTEID INJECTED, RECOVERED AND RETAINED.—Continued.

Experiment number.	Other experiments done on same animal.	Animal.	Weight in kilos.	Manner of injection.	Solution used.	Absolute amount of proteid injected	Absolute amount of proteid recovered.	Absolute amount of proteid retained.	Amount of proteid injected per kilo. of animal.
XXIII A	None.	Guinea.	0.275	Hypodermic.	Egg, watery.	0.3+ in 2 days.			
B	"	"	0.245	"	" "	" "			
XXIV A	(XXVIII).	Rabbit ♂	2.26	Ear-vein.	" "	0.156	Too small to be accurate.		0.069
B	"	" ♀	2.25	"	" "	0.281	Too small to be accurate.		0.125
XXV A	None.	Guinea	0.485	Hypodermic.	" "	1st day 0.034			In 12 days
B	"	"	0.230			3d " 0.031			A 0.307
						12th " 0.084			B 0.648
					Each				
						Total in 12 days 0.149			
XXVI	"	Dog ♂	5.9	Vein.	" " 3.402%	3.402	[4.662 in 1 hr. 40 min.]		0.576
XXVII	"	" ♂	7.0	"	" " 3.4%	5.1			0.729
XXVIII A	(XXIV) 8 days after previous injection.	Rabbit ♂	2.21	Hypodermic.	" " 8.362%	6.469	0.963 +	Died before complete excretion.	2.927
B		" ♀	2.05	Peritoneum.	" " "	5.850	0.931 +		2.853
XXIX	None.	Dog ♂	5.8	Vein.	" norm. salt 3.397%	5.096			0.875
XXX A	"	Guinea pig	0.33	Peritoneum.	" " "	A. B.			In 8 days.
B	"	" "	0.25			1st day 0.203 0.203			A 8.119
						4th day. 0.763 0.636			
						7th day. 0.569 0.569			B 10.184
						8th day. 1.138 1.138			
					Total in 8 days.	2.673 2.546			
XXXI	"	Rooster		"	" norm. salt.	0.763	None.		
XXXII	"	Rabbit ♂	1.21	Vein.	" " heated to 73°, 1.255%.	1.673	[0.6174 in 1 hr.]		1.382
XXXIII	"	" "	1.79	"	Human muscle in normal salt 0.389%.	0.486	None in 5 hours.		0.272
XXXIV	"	Hen		"	Egg, watery.	Dies at end of injection.			
XXXV	"	"		"	" "	Dies as wound is sewn up.			
XXXVI	"	"		Peritoneum.	" " 4.5%.	2.275			
XXXVII A	"	Rabbit ♀	0.975	"	" "	1st day 4.31	0.367	1.908	
						5th " 3.5	0.915	3.395	4.42
B	"	" "	0.185	"	" "	1st day 4.31	0.794	2.706	3.59
						5th " 3.5	1.387	2.923	3.64
XXXVIII A	"	" ♂	1.140	"	Chicken muscle in	4.375	1.112	2.388	2.95
B	"	" "	1.220	"	normal salt 4.375%.	"	Died.		3.83
XXXIX A	"	" "	0.973	"	Dog's muscle norm. salt.		None.	All.	3.74
B	"	" "	0.905	"	" " " "		None.	All.	
XL	"	Dog ♂	3.75	Hypodermic.	Egg, watery.	0.469	Died.		
XLI	"	" ♀	3.	Vein.	Egg with equal vol. norm. salt.	100 cc.	None.	0.46	0.125
XLIV	"	Rabbit	1.570	"	Dog's muscle in norm. salt.	100 cc.	Dies at end of injection.		
							None.	All.	

TABLE II.—CONTINUATION OF EXPERIMENTS I–XLIV. PERCENTAGES OF PROTEID RETAINED AND EXCRETED. DURATION OF EXCRETION. EXAMINATION OF URINE.

Experiment No.	Amount of proteid retained per kilo.	Per cent. of the injected proteid excreted.	Per cent. retained.	Per cent. excreted in first 24 hours.	Excretion lasted between—	Special remarks on urine.	Glycosuria.
I	0.062	66.67	33.33		42 and 55 hours.		1.3 gm.
II					2 and 5 days.	Non-coagulable proteid.	None.
III							1.956
IV		67+			Over 3 days.		None.
V	0.133	65.07	34.93	19.5	43 and 64 hours.	Small amount of globulin.	"
VI			...		40 hours.		"
VII	0.314	0	100	0	0	No casts.	"
IX						Contains haemoglobin and globulin, probably from admixture of wound secretion, since this kept open.	"
X	0.433	32.	68		55 and 71 hours.	No globulin.	"
XI	0.615	0	100	0	0		...
XII	0.021	11	89		2 and 3 days.	No casts.	None.
XIII					11 days after last injection.	" "	"
XIV	...						"
XV		87+					"
XVI	0.427	38.9	61.1	38.1	24 and 42 hours.		
XVIII	0.279	55.7	44.3	42.	90 hours.	Trace globulin.	None.
XIX		55+	...	40.5	42 hours.		Large amount.
XX				167.	10 days+.		Fair amount.
XXI				125.	5 days.		None 1st day, some 2d, none after, but plenty on morphine.
XXII				[75 in $2\frac{1}{2}$ hours.]		Much globulin.	None.
XXIV A and B		.. .				No albumose.	"
XXVI	...	...		[137 in 1 hr. 40 min.]		Much globulin.	"
XXVIII A		14.9+		5.1	3 days+.	Considerable albumose.	"
" B		15.9+		13.9	3 days+.	" "	"
XXIX				.. .	.. .		
XXXII			...	[36.8 in 1 hour.]		No globulin; no precipitate below 73°.	
XXXIII						Proteid free.	
XXXVI		16.1	83.9	16.1	24 hours.	No globulin.	
XXXVII A	3.482	21.2	78.8	10.3	3 days.	Albumose increased.	
" "	2.775	22.7	77.3	12.7	4 days.	" "	
" B	2.467	32.2	67.8	17.4	2 days.	" "	
" "	2.015	31.8	68.2	8.1	3 days.	" "	
XXXVIII A and B							None.
XL	0.125	0	100	0	0	No albumose.	"
XLIV		0	100	0	0		"

TABLE III.—CONTINUATION OF EXPERIMENTS I-XLIV. SYMPTOMS AND AUTOPSIES.

Experiment number.	Time of death.	Symptoms.	Autopsy.	Special Observations.
I		Stupid next day, lively on 2d.		Dinresis.
II		None.		
III		Steadily emaciating since beginning of experiment, but voracious appetite.		Normal saline on sugar.
IV	3 days after last injection.	Greatly depressed next day, can scarcely walk on 3d. Temperature 39.5° C.	Extensive pyothorax; acute congestion of kidneys; heart muscle normal.	
V	Killed 5th day.	None. General condition good.	Slight cortical congestion of kidneys, particularly Malpighian tufts; cells granular.	
VI	7 hours.	Coma and convulsions; bloody diarrhœa.	Hæmorrhage, congestion and necrosis of abdominal organs; blood changes. See p. 251.	Peculiar toxin action.
VII		None.		Excretion native albumen after injection of alkali albumen.
X	Killed on 3d day after last injection.	None. Good condition.	Congestion of abdominal organs, particularly cortex of kidneys.	
XI	Killed on 2d day after last injection.	Almost in stupor.	Kidneys mislaid; other organs practically normal.	
XII	Killed on 7th day after last injection.	None.	Negative.	Transitory albuminuria 4 days after injection.
XIII	About 40th day.	Emaciation.	Not made.	Long continued excretion.
XIV	4 hours.	Progressive medullary paralysis	Negative.	Death probably from embolism; injection of mixture of alkali and albumen.
XV	16 hours.	Not known.	Negative, but stomach distended with 10 ounces (=10% body weight) of semi-solid food.	Shock during full digestion?
XVI				Coagulation temp. of urine.
XVII	15 minutes.	Respiratory, then cardiac paralysis.		Death by embolism.
XVIII	4 days.	None till last hour, then convulsive. See p. 249.	Negative. Microscopically congestion of abdominal organs.	Death under convulsions after 4 days of health.
XIX	40 hours.	Depression, anorexia. See p. 247.	Not made.	
XX		None.		Nephritic albuminuria.
XXI	1 day after morphine.	None.		"
XXII				Comparison of fractional heat precipitates. Physiological effects.
XXIII A	28 hrs. after second injection.	None for 6 hrs., then depressed.	Much light red fluid in subcutaneous tissue and abdominal muscles. Otherwise negative; kidneys trifle congested.	Toxic effects.

TABLE III.—CONTINUATION OF EXPERIMENTS I-XLIV. SYMPTOMS AND AUTOPSIES.—Continued.

Experiment number.	Time of death.	Symptoms.	Autopsy.	Special Observations.
XXIII B	10 hrs. after second injection.	None for 6 hrs., later not observed.	Same as XXIII A.	Toxic effects.
XXIV A & B.				Metabolism.
XXV A		Local ulcers which heal. No other effect.		No toxic effect.
B		None.		" "
XXVI				Comparison of fractional heat precipitates. Physiological effects.
XXVII		Complete anuria for 4 hours, although 400 cc. of liquid (=56 cc. per kg.) were injected.		
XXVIII A & B.	3 days.	Debility and emaciation.		Starvation and metabolism.
XXIX				Comparison fractional heat precipitates.
XXX A & B.	About 14 hrs. after last injection.	Some depression.	Free fluid in peritoneum; else negative.	Toxic effects.
XXXI		None.		Rooster. None excreted.
XXXII				Inject. of albumen heated to 73.
XXXIII	5 hours.	Diarrhœa, coma, fall of temperature, respiratory paralysis. See p 247.	Congestion of abdominal organs; degeneration of liver cells.	Muscle inject'n; none excreted. Toxic effects (anæsthetic).
XXXVI				Excretion egg albumen by hen.
XXXVII A & B		None.		Metabolism.
XXXVIII A	Between 5 and 15 hours.	Not observed.	Negative.	Toxicity of chicken muscle.
B.		None.		Retention of chicken muscle.
XXXIX A		None.		Retention of dog's muscle.
B	Between 5 and 15 hours.	Not observed.	Negative.	Toxicity of dog's muscle.
XL		None.		Metabolism.
XLI	10 minutes.	Paralysis of respir'n and heart.		Embolism.
XLIV	Killed on 3d day.	Fever.	Negative.	Myosin. Fever.

TABLE IV.—SUMMARIES OF EXPERIMENTS 43 TO 72.

No.	Animal.	Anaesthetic.	Experiment.	Fate.	Special Remarks.	Histological Findings.	Distribution of Pigment.	Stain for Iron.
43	Rabbit, 950 gm.	Urethane, 2.5 gm. stomach.	Intravenous injection.	Died during injection.	Acute Urethane death.			
44	Rabbit, 1570 gm.	Chloretone, 25 cc. sat. sol. stomach.	100cc. Dog's muscle solu. intravenously.	Killed with chloretone on 3d day.	Muscle and death by chloretone.	*Kidney:* normal. *Liver:* considerable fatty degeneration of epithelium. *Spleen:* normal.	*Liver:* marked in places. *Spleen:* a few pigment bearing cells.	*Liver:* none. *Spleen:* quite profuse.
45	Rabbit.	As 44.	No injection.	Died acutely.	Acute chloretone death.	*Liver:* marked congestion; cells unaltered. *Spleen:* normal.	*Spleen:* slight.	*Liver:* none.
46	Rabbit.	As 44.	As 45.	Died in 3 to 16 hours.	Subacute chloretone death.	*Kidney:* greatly congested. *Liver:* much capillary congestion. Cells generally unaltered. *Spleen:* normal.	*Liver:* some.	*Liver:* none.
47	Rabbit, ♂960 gm	Urethane, 1 gm. rectum.	Intravenous injection.	Died in 1½ hrs.	Acute urethane death.	*Kidney:* considerably congested. Cells granular. *Liver:* cells very much vacuolated and granular.	None in liver or kidney.	
48	Rabbit, 850 gm.	As 47.	" "	Died in 3½ hrs.	Subacute urethane death. Urine contained very many granular casts and some blood. Large quantity of free blood in abdomen; clots on removal. (Injury during autopsy ?)	*Kidney:* cells considerably degenerated. *Liver:* cells greatly vacuolated and fatty.	*Kidney:* Pigment in the epithelium of the convoluted tubules (iron free.) *Liver:* some.	*Kidney:* none.
49	Rabbit, 1310 gm.	As 47.	15 cc. 5 day brooded egg, into jugular.	Died in 5 days.		*Kidney:* granular degeneration of epithelium. *Liver:* extensive capillary congestion. Thrombi in many veins and capillaries.	*Kidney:* in epithelium. *Spleen:* considerable; rather diffuse.	*Kidney:* none. *Spleen:* great amount.
50	4 guinea-pigs.		Each received 1 cc. of 1 day brooded egg solution subcutaneously.	One dies in 5 days.				
51 A 53 B 57 D	Rabbit, 1050 gm. 1070 " 970 "	Urethane, 0.75 gm. rectum.	15cc. (=0.362 gms.) of fresh egg of brooding series. *7 days later*, 11cc. (=0.254 gm.) of 6 day brooded egg into ear vein. *6 days later*, normal salt, subcutaneously.	Killed by medulla stroke, 2 days after last experiment.		*Kidney:* some congestion; cells rather cloudy; no casts. *Liver:* proliferation of young connective tissue about lobules; cells granular. *Small Intestine:* slightly congested. *Cardiac muscle* and *large intestine:* normal.	*Kidney:* considerable in epithelium of convoluted tubules. *Liver:* small amount.	*Kidney* and *Liver:* none.

TABLE IV.—SUMMARIES OF EXPERIMENTS 43 TO 72.—Continued.

No.	Animal.	Anaesthetic.	Experiment.	Fate.	Special Remarks.	Histological Findings.	Distribution of Pigment.	Stain for Iron.
51 B 54 A	Rabbit, 1000 gm. 880 gm.	Urethane, 0.75 gm. per rectum.	15 cc. (= 0.362 gm.) of fresh egg of brooding series. 7 *days later:* norm. salt. ear.	Died 7 days after last experiment.		*Kidney:* considerable congestion; cells very granular. *Liver:* congested; cells somewhat cloudy. *Spleen, cardiac muscle* and *lungs:* normal. *Hyperleucocytosis* in blood of lungs and kidneys, not noticed in other situations.	Small amount in *Spleen.* None in *lungs, cardiac muscle, liver* or *kidney.*	*Spleen:* some; but most of the pigment does not take it.
51 C 53 A 56 A 57 A	Rabbit, 1000 gm. 960 " 1080 " 1050 "	Urethane, 0.5 gm. per rectum.	10 cc. (=0.24 gm.) fresh egg sol. of brooding series, in ear vein. 10 *days later:* norm. salt in ear vein. 2 *days later:* 15 cc. egg soln. from 9 day brood. 4 *days later:* norm. salt intraperitoneally.	Killed by medulla stroke, 8 days after last injection.		Bacterial infection profound.	*Liver:* considerable amount of yellow granules in cells. *Spleen:* large amount, granular and in lumps. *Kidney* and *cardiac muscle:* none.	*Liver:* none. *Spleen:* large amount; practically all the pigment has taken the stain.
52 A	Rabbit, 1500 gm.	Urethane, 1 gm. per rectum.	20 cc. (= 0.411 gm.) egg soln. of 3 days brood, jugular.	Died in 6 days from starvation.				
52 B 55 A	Rabbit, 1030 gm. 980 gm.	Urethane, 0.5 gm. per rectum.	6 cc. (=0.123 gm. egg soln. of 3 days brood, ear vein. 6 *days later:* 15 cc. alkaline egg syntonin soln. by jugular.	Died in 2 days after last experiment.	Death from bacterial invasion.	Urine contained a few granular casts. Profound bacterial infection.	*Liver:* only in hæmorrhagic areas. *Spleen:* none.	*Spleen:* diffuse light blue stain. *Liver* or *Kidney:* none.
53 C 56 B 57 C	Rabbit, 1080 gm. 1070 gm. 1060 "	Urethane, 0.5 gm. per rectum.	15 cc. (=0.347 gm.) egg soln. of 6 days brood, jugular. 2 *days later:* 6 cc. alkaline egg syntonin soln., ear vein. 4 *days later:* normal salt subcutaneously.	Killed by medulla stroke, 30 days after last experiment.		Strong bacterial infection.	*Spleen:* considerable. *Liver:* doubtful. *Kidney* and *cardiac muscle:* none.	*Spleen:* very slight.
55 C	Dog 4.3 Kg.	Morphine, ether.	25 cc. alkaline egg syntonin soln. femoral.	Killed by heart puncture in 2 days.		*Kidney:* Cells granular; great degeneration in limited areas; casts. *Liver:* slight cellular degeneration. *Suprarenal, spleen, pancreas, small intestine, and cardiac muscle:* all normal.	*Spleen:* some in granules and lumps. *Suprarenal:* some granules in cortex, normal pigmented cells in medulla. *Liver, kidney,* all kinds of *muscle:* none.	*Spleen:* considerable. *Kidney* or *intestine:* none. *Liver:* diffuse light stain of cells, but granules only in thrombus.
55 B 57 B	Rabbit, 1030 gm. 1060 gm.		10 cc. egg soln. of 9 days brood, ear vein. 4 *days later:* norm. salt intraperitoneally.	Died 16 days after last experiment.		Strong bacterial infection.	*Spleen:* considerable. *Liver:* some. *Kidney* and *cardiac muscle:* none.	*Spleen:* very slight.

TABLE IV.—SUMMARIES OF EXPERIMENTS 43 TO 72.—Continued.

No.	Animal.	Anaesthetic.	Experiment.	Fate.	Special Remarks.	Histological Findings.	Distribution of Pigment.	Stain for Iron.
60	Rabbit, 785 gm.		6 cc. norm. salt, in ear vein.	Killed by medulla stroke in 3 days.		Slight bacterial infection.	*Kidney* or *liver*: none. *Spleen*: considerable amount, granular and clumps.	*Spleen*: very slight and diffuse.
61	Rabbit, 834 gm.		8 cc. norm. salt, in ear vein.	As 60.		As 60.	Little in *spleen* or *suprarenal*; a few granules in *liver-cells*. None in *kidney*.	*Spleen*: very little. *Liver*: none.
62	Rabbit, 885 gm.	Urethane, 0.5 gm, per rectum.	25 cc. alkaline egg syntonin into jugular.	Killed by medulla stroke in 10 days,	Long fever.	*Kidney*: Rather slight granular swelling of epithelium, considerable congestion of capillaries and tufts. *Liver*: considerable capillary congestion; cells normal. *Spleen* and *suprarenal* normal	*Spleen*: considerable, granular and in lumps. *Liver*: ditto: *Kidney*: none.	*Spleen*: very large amount. *Liver*: none.
63	Rabbit, 1180 gm.		1 cc. alkaline egg syntonin in ear vein.	Killed by medulla stroke in 3 days.		Moderate bacterial infection.	*Spleen*: considerable, *Liver*: rather large amount of granular.	*Spleen*: considerable. *Liver*: none.
64	Rabbit, 1195 gm.	Urethane, 0.5 gm. per rectum.	Died on table.			Strong bacterial infection.	*Spleen*: little. *Liver*: much, granular.	*Spleen*: weak, diffuse. *Liver*: a little in spots, diffuse and coarsely granular.
65	Rabbit, 1225 gm.	As 64.	20 cc. alkaline egg syntonin, jugular.	As 63.		Very slight bacterial infection: *Kidneys*: hyaline degeneration in cells of convoluted tubules. *Liver*: diffuse granular degeneration of cells. *Spleen*: normal.	*Kidney*: many granules in epithelium. *Liver*: considerable free granules. *Spleen*: small.	*Liver*: none. *Spleen*: considerable.
65 A	Dog.	Morphine-ether.	50 cc. alkaline egg syntonin, femoral.	Killed in 3 days.		Congestion of *abdominal viscera*. *Small intestine*: degeneration of epithelium of villi. *Kidney*: extensive hyaline degeneration and cloudy swelling. *Liver*: epithelium normal, also *suprarenals* and *large intestine*.	*Spleen*: considerable, in clumps. *Intestine*, *liver* and *kidney*: none. *Suprarenal cortex*: slight.	*Spleen*: considerable. *Liver*: none. *Kidney*: some in isolated places in cells.

TABLE IV.—SUMMARIES OF EXPERIMENTS 43 TO 72.—Continued.

No.	Animal.	Anaesthetic.	Experiment.	Fate.	Special Remarks.	Histological Findings.	Distribution of Pigment.	Stain for Iron.
66	Rabbit, 1255 gm.	. ..	2 cc. sol. of rabbit's muscle, in ear.	Killed by medulla stroke in 3 days.	Few bacteria.	*Kidney:* Cells as in 65, but less. *Liver:* considerable cellular infiltration of perilobular tissue. Some congestion, especially of central veins. Blood appears to contain large number of leucocytes. Cells normal. *Spleen* and *pancreas:* normal.	*Spleen:* small amount. *Liver* and *kidney:* considerable.	*Spleen:* small amount. *Liver* and *kidney:* none.
67	Rabbit, 1380 gm.		1 cc. as 66.	As 66.		*Kidney, liver, spleen* and *suprarenal* are all normal.	*Spleen:* very little. *Liver:* some granules in cells. *Kidney* and cortex of *suprarenals:* none.	*Spleen:* almost none. *Liver:* none.
68	Rabbit, 1375 gm.	0.5 gm. urethane, per rectum.	25 cc. soln. of rabbit's muscle, jugular.	As 66.		*Kidney:* extensive cloudy swelling of epithelium of convoluted tubules. Hyaline casts. *Liver:* slightly fatty, otherwise normal. *Spleen* and *Suprarenal:* normal.	*Spleen* and *liver:* considerably granular. *Suprarenal* and *kidney:* none.	*Spleen:* considerable. *Liver:* none.
69	Rabbit, 1435 gm.	As 68.	As 68.	Killed by medulla stroke 10 days.		*Kidney:* some cloudy swelling and vacuolization of epithelium. *Liver, spleen, suprarenal:* normal.	*Spleen:* very little. *Liver:* considerably granular in cells. *Suprarenal cortex:* none.	*Liver:* and *spleen:* none.
70	Rabbit.			Killed by medulla stroke.	Normal.	*Kidney:* considerable cloudy swelling of the epithelium of the convoluted tubules. *Liver:* slight congestion, with extensive granular degeneration of cells.	*Spleen:* almost none. *Liver* and *Kidney:* none.	*Spleen:* almost none.
71	Rabbit, 730 gm.	0.5 gm. urethane by rectum.		Killed by medulla stroke in 4 days.	Urethane alone.	*Kidney:* very slight degeneration and vacuolization. *Liver:* slight capillary congestion. Cells normal.	*Kidney:* none. *Liver:* slight.	*Liver:* none.
72	Rabbit, 870 gm.	As 71.		Killed by medulla stroke in 7 hours.	As 71.	*Kidney:* some cloudy swelling. *Liver:* extensive granular degeneration, as 70. *Large intestine:* normal.	*Spleen:* considerable. *Kidney* and *liver:* none.	*Spleen:* considerable diffuse pigmentation.

One of us (Sollmann) is responsible for experiments I to XXI, and 44 to 72. The others were done by us jointly. We take this opportunity of acknowledging our obligations to Professor G. N. Stewart, who first directed our attention to this subject, and in whose laboratory the first experiments were made. We are also greatly indebted to Professor Wm. T. Howard, Jr., for the preparation and description of the greater part of the histological material.

II. METHODS.

A. Operation.

A known quantity of egg-albumen, obtained by dissolving egg white in distilled water or normal salt solution (and always containing a small quantity of egg-globulin), was injected under anæsthesia into the femoral vein (dog) or into the jugular (rabbit); or without anæsthesia into the ear vein or intraperitoneally or hypodermatically, and the urine was collected by the methods presently to be described. The dogs were anæsthetized with morphine and ether, using the smallest quantity of the latter which would keep the animal quiet. To insure against albuminuria from the anæsthetic, the urine was drawn and analyzed immediately before injection. The absence of a notable quantity of globulin in typical experiments is also an indication that in these no part of the albuminuria resulted from the anæsthetic. The anæsthesia was performed on rabbits by injecting through a catheter a solution of chloretone into the stomach, or urethane into the rectum. The amounts are noted in the foregoing tables. The injection of the proteid solution was made fairly rapidly through a burette connected with the femoral vein by a glass cannula. It was aimed to make the operation aseptic, but in the case of the dogs we were not uniformly successful in this respect.

The method of collecting the urine differed with the experiment. After intravenous and intraperitoneal injection, the animals were placed in a zinc-lined or galvanized-iron cage, with an opening for the collection of the urine. The cage was washed daily with distilled water and the washings placed with the urine; it was then flushed with a small quantity of saturated boric acid solution, and some of this was also placed in the collecting vessel. The urine collected in this way showed no trace of decomposition. In the metabolism experiments, the animals were catheterized daily and their bladders rinsed with distilled water. These animals were fed purely on oats and water. The dogs received their customary diet of meat, bread and water.

With intravenous injection, the dogs (female) were catheterized before the injection was made, and the catheter was left in position for an hour or two after the injection; the animals being kept on the operating board, lightly under the influence of ether. The wound was then stitched and covered with collodion and the animals were placed in cages for the further collection of urine. In the experiments in which it was desired to know only the ratio of the excretion of the different proteids, thin glass tubes were tied into the ureters and the urine was collected directly from these, the animal in this case not being allowed to recover from the anæsthetic.

The same animals were sometimes used a second time after they had completely recovered from the effect of the first injection. When an animal died, a careful autopsy was made and the kidneys were examined histologically after hardening and sometimes also in the fresh condition. In certain cases, other organs were also preserved in Orth's fluid, sectioned, and stained with hæmatoxylin, eosin, and for iron.

B. Analytical Methods.

In a majority of the experiments we directed our attention mainly to the total quantity of proteids, but tested in each of experiments I-XLIV for sugar (Trommer's test) and for non-coagulable proteid (ferrocyanide reaction in the absence of coagulable proteid). For further analysis the urine, if not already acid, was treated with 1% acetic acid until it just reddened litmus paper. It was then filtered and the coagulable proteid determined gravimetrically in a definite volume (10-100 cc.). The same method was used for the albumen solution. The urines of experiments I-XLIV were always tested qualitatively and often quantitatively for globulins by Pohl's method (half-saturated sol. ammonium sulphate).

In the experiments in which we intended to weigh separately the precipitates occurring at different temperatures, we heated the urines slowly in a large water-bath and kept them at the desired temperature for ½ hour. If the quantity of material admitted, a separate sample was taken for each temperature. In the observations on metabolism, where we wished to determine the quantity of the various nitrogenous constituents, the small amount of material and the numerous determinations to be made rendered it necessary to institute some preliminary experiments for the selection of a suitable method of analysis.

Preliminary Experiments for the Selection of a Method of Analysis.

1. *Determination of Urea.*—The separation of the different nitrogenous constituents was effected in the first place by phosphotungstic acid in hy-

drochloric solution...As has been shown by various experimenters, the precipitation of nitrogenous bodies by this substance is not perfectly rigorous. The amount of precipitation varies with the time during which the reagent is allowed to act, with the sample of the reagent and with the dilution. The following results are given by the different observers who have investigated this subject. Our own observations are included in this compilation:

TABLE V.—PRECIPITATION OF NITROGENOUS SUBSTANCES BY PHOSPHOTUNGSTIC ACID.

[Where a space is left blank in the table, the substance has not been investigated].

	Mallet (1).*	Reid (2).*	Schöndorff (3).*	Sollmann and Brown.
Precipitated—				
Egg albumen	Precipitate, insoluble in hot water.			Prec. complete.
Serum proteid	"			
Myosin	"		Prec. complete.	
Vitellin	"			
Syntonin	"			
Casein	"			
Legumin	"			
Fibrin	"			
Haemoglobin	"			
Albumoses	"	80 to 90% prec.		Prec. complete.
Gelatin	"			
Choudrin	"			
Xanthin series		Precipitate.		
Xanthin				Prec. complete.
Uric acid			Prec. complete.	" "
Caffein			" "	
Guanin			" "	
Lysin		"	" "	
Creatinin	"	"		
Ammon. sulphate			" "	Precipitate 87%
				Used Schuchardt's acid. Kahlbaum's precipitates only partially, Merck's completely (Pfandler).
Creatin	Prec. soluble in hot water.	0	0	
Peptone	"			
Glutamin	"			
Betain	"			
Hypoxanthin	"			
Carnin				
Not precipitated—				
Urea	"	0	0 (absolutely)	0 (absolutely)
Alanin	0		0	
Allantoin	0		0	
Alloxantin			0	
Asparagin	0			
Aspartic acid	0	0	0	
Glycocoll			0	
Glycosin	0	0		
Glutamic acid	0	0		
Leucin	0	0	0	
Sarcosin			0	
Taurin			0	
Tyrosin	0	0	0	

*These numbers refer to the bibliography at the end of this article.

Phosphotungstic acid allows us to separate therefore:

In solution: Urea, amido-acids and peptones. Since the last two exist only in traces, the filtrate may practically be interpreted as urea.

In precipitate: Proteids (coagulable and albumoses), uric acid, xanthin bodies and ammonia.

The following protocols give the experiments made to test the method. The detail of the tests are the same as given below (p. 222).

1. Phosphotungstic acid + egg-albumen = Precipitate (filtrate free from biuret.)
 " " + Peptone (Witte) = Precipitate.
 " " + Sodium Urate = "
 " " + Ammon. Sulphate = "
 " " + Urea = no precipitate.
2. Urea: the filtrate yields 87% of the calculated nitrogen.
3. Ammonium Sulphate: the filtrate yields 13% of the calculated nitrogen of the ammonia.
4. Mixture of urea, ammonium sulphate, sodium urate and peptone (Witte). The filtrate shows 104% of the urea.
5. Mixture of urea, ammonium sulphate, sodium urate and peptone (Witte). The filtrate shows 114% of the urea.

It will be seen that the figures for the urea are somewhat in excess.

2. *Determination of Ammonia.*—This was done by Schloesing's Method, which was tested as follows:

1. Parallel determinations on ammon. sulphate solutions give:

Desiccation	1.000
Distillation with sodium hydrate	0.925
Sulphuric acid by barium	0.977
Schloesing	0.905
Hypobromite	0.778 *

2. Schloesing's method on a mixture of ammon. sulphate, urea, sodium urate, peptone (Witte), and coagulable proteid yields 103% of the ammonia.
3. Schloesing's method on another similar mixture yields 107% of the ammonia.

The method gives slightly excessive results.

3. *Determination of Coagulable Proteids.*—This was done by heating the slightly acidulated urine with sodium sulphate to boiling, and then adding a few drops of ferric acetate. A Kjeldahl determination was then done on the precipitate and filter, the nitrogen of the filter being subtracted.

Ferric acetate gives no precipitate with sodium urate or Witte's peptone.

The difference obtained by subtracting the nitrogen of the urea, ammonia and coagulable proteid from the total nitrogen represents the nitrogen of albumoses, xanthin bodies and uric acid, minus the somewhat variable factor of error in the above determinations.

To eliminate this uncertain quantity and to obtain a further insight into the make-up of this rest we tried various methods of directly esti-

*The decomposition with hypobromite was undertaken with a view of utilizing this method for the determination of the ammonia. It is seen that the result is unreliable.

mating these constituents; but, it must be confessed, with indifferent success.

Our experiments were restricted to such quantities and concentrations as we could expect in rabbit's urine, and we cannot make any statements concerning the usefulness of these methods when employed under other conditions.

4. *Determination of Albumoses.*—We tried the bromine and zinc-sulphate methods (for details see below). The following are our results:

(*a*) *Bromine Method* (Wiley) (4): 1. Mixture of Witte's peptone and uric acid yields 74 per cent of the albumose.

2. Mixture of Witte's peptone, egg-albumen, sodium urate, urea and ammonium sulphate yields 79 per cent of the albumose.

Whilst the method is inaccurate, the comparative values obtained by it are probably useful.

b) *Zinc-Sulphate Method.*—This was tried only in the last series of experiments, but gave the most promising results of all:

1. Peptone (Witte): Completely precipitated; no biuret in filtrate.
 Egg-albumen: Completely precipitated; no biuret in filtrate.
2. Sodium urate, Urea, } no precipitate.

(*c*) *Almen's tannin mixture* gave the following results:

1. Sodium urate: Precipitated 71 per cent of the uric acid.
 Ammonium sulphate and urea: No precipitate.
2. Sodium urate, Peptone (Witte), Egg-albumen, } were mixed with urea and ammonium sulphate. The precipitate with Almen's mixture contained but 30 per cent of their nitrogen.

This reagent is therefore not applicable.

The methods of directly estimating the alloxur bodies and uric acid appear to require much larger quantities of urine than we had at our disposal, and demand so much time that we did not give them a thorough trial.

The *methods* finally adopted are the following:

1. *Total nitrogen,* by Kjeldahl: 2 to 5 cc. of urine are placed in a 250 cc. flask with 20 cc. of concentrated sulphuric acid, mercury being added in just sufficient amount to aid oxidation, and the mixture boiled until almost colorless. The flask is allowed to cool and the contents rinsed into a litre flask with three portions of distilled water, of about 75 cc. each. A few pieces of metallic granulated zinc are then added, followed quickly by 70 cc. of a 40% (by weight) solution of caustic soda and 10 cc. of 40% solution of sulphuret of potash. The flask is quickly connected with a Liebig condensor, and heated until the distillate is free from ammonia. The distillate is received in a flask fitted air tight to the condensor. This flask contains a measured amount of decinormal sulphuric acid, and is also connected with a U-shaped absorption tube charged with the same. The distillate is then titrated, methyl orange (U. S. P.) being used as an indicator.

A blank determination was made on every new lot of chemicals and corrections applied if necessary.

Duplicate determinations were made in many cases, but not in all.

2. *Phosphotungstic Filtrate.*—A solution of phosphotungstic acid (100 ccm. phosphotungstic acid (Schuchard) to 800 cc. of 4% hydrochloric acid) is added to 5-20 cc. of urine diluted to about 30 cc., until no further precipitate occurs, then made up to 50 cc. and allowed to stand from one to three hours, filtered, and the nitrogen determined as above on 20 or 25 cc. of the filtrate. The result gives the nitrogen of *urea.*

3. *Ammonia* (Schloesing).—20-50 cc. of urine are mixed with 10-20 cc. of milk of lime and placed in a desiccator with a porcelain capsule which contains 10-20 cc. of decinormal sulphuric acid. This is allowed to stand for five days and the excess of acid titrated. The result gives nitrogen of *ammonia.*

4. *Coagulable Proteids.*—50-150 cc. of urine are rendered faintly acid with acetic acid, mixed with an equal volume of 10% sodium sulphate and boiled. When near the boiling point a few drops of ferric acetate are added. This is filtered, the precipitate washed until free from sulphates, and the nitrogen determined in the filter and precipitate. The amount of nitrogen contained in the filter is determined once for all and subtracted. This procedure was used only for the urines collected after injection.

5. *Albumoses.—a. Bromine method.*—25 cc. of urine are rendered strongly acid by concentrated hydrochloric acid. 2 cc. of bromine are added, the mixture is shaken, stoppered tightly, and left to stand over night. Some undissolved bromine must remain in the flask. The mixture is then filtered, and the precipitate washed by decantation. The filter is then returned to the flask and subjected to a Kjeldahl determination. Subtraction of the nitrogen of the filter gives the nitrogen of the total proteids. Subtraction from this of the nitrogen of the coagulable proteids leaves that of non-coagulable proteids.

b. Zinc-sulphate method.—25 cc. of urine are saturated with 35 grm. of crystalline zinc sulphate,* and filtered; the precipitate is washed with saturated solution of zinc sulphate and subjected to Kjeldahl, as in (*a*).†

* It is necessary to use tested zinc sulphate. One commercial sample yielded 2.1 mg. nitrogen per grm., or in a test carried out as above, about 1 mg. for the 25 cc. of urine.

† According to Bömer, it would have been better to acidulate the urine, and the saturated zinc sulphate solution with 2 cc. per 100 cc. of 1:5 sulphuric acid.

By these methods we could determine the following data:

(a) *Total nitrogen*—Method 1.—Exact.

(b) *Urea nitrogen*—Method 2.—Somewhat in excess.

(c) *Non-urea nitrogen*—(a minus b).—Too low.

(d) $\frac{\textit{Urea nitrogen}}{\textit{Non-urea nitrogen}} = \frac{b}{c}$—Somewhat in excess.

(e) *Ammonia*—Method 3.—Slight excess.

(f) *Coagulable proteids*—Method 4.—If anything, slight excess.

(g) *Non-coagulable proteids*—Method 5 minus (f).

(h) *Alloxur and uric acid* = a—(b + e + f + g).—Open to too many sources of error to be very reliable, but still of limited value.

III. EXCRETION OF THE PROTEID.

We shall discuss this subject under the following headings:

A. Proportion of the coagulable proteid excreted.

B. What determines the quantity of albumen retained.

C. Nature of the excreted proteid.

D. Excretion of the different constituents of egg-albumen.

E. Duration of the albuminuria.

F. Beginning of the excretion of albumen.

G. Relative quantity excreted in successive periods.

H. Retention of other proteids after intravenous or subcutaneous injection.

A. Proportion of the Coagulable Proteid Excreted.

Older observers, working mainly with qualitative methods, claim that injected egg-albumen is excreted unchanged, almost in its entirety. Thus Stokvis (5) states that egg-albumen given hypodermically "stellt eine durchaus unbrauchbare Substanz dar." But all later experimenters who have investigated this subject quantitatively, agree that this excretion is generally far from complete.

Observations on the quantitative excretion of proteid indicate the need of special precautions to guard against nephritic albuminuria. We have encountered this condition not infrequently. Excluding, therefore, all cases in which the amount excreted exceeded that injected; those in which the albuminuria continued indefinitely; those in which the urine contained considerable globulin; those in which it showed blood-pigment; and finally also those in which the animal died

before the excretion had ceased, we can present the following results bearing on this question:

After hypodermic injection of very small quantities (the largest 0.125 grm. per kilo. body weight) into either dogs or rabbits, none of the proteid is excreted:

Dogs, hypodermic injection, 0.02 to 0.43 grm. per kg., two cases, retained 68 and 89%.

Dogs, intravenous injection, 0.19 to 0.7 grm. per kg., four cases, retained 33 to 61%; mean, 40%.

Rabbits, intraperitoneal injection, 3 to 4.4 grm. per kg., four cases, retained 66 to 80%; mean, 72%.

The following cases were found in the literature:

Forster (6): Dog, intravenous, large quantity, retained 27.4%.

Lehmann (7): Dog, intravenous, small quantity, retained 23%.

Munk and Lewandowsky (8): Dog, slow intravenous, very small quantity, retained 82%; rabbit, slow intravenous, retained 54%: rabbit, intraperitoneal, retained 68%.

Adding these to ours, we see that, when moderate to large quantities are injected, the *retention* is as follows:

	Dog, intravenous.	Dog, hypodermic.	Rabbit, intravenous.	Rabbit, intraperitoneal.
Number of cases.....	7	2	1	5
Extremes	23 to 82%	68 to 89%	54%	66 to 80%
Means	35%	78%		71%

B. What Determines the quantity of Albumen retained?

From the results given in the preceding section, it is seen that the retention varies from 23 to 100% of the amount injected. It is likewise evident that:

1. Very small quantities are retained completely, at least when given hypodermically.

2. With very large quantities (Forster's case) a larger absolute amount is retained, but the proportion of that retained to that excreted is smaller.

3. The greatest retention occurs on hypodermic injection. Less is retained on intraperitoneal, and least on intravenous injection.

From 3 we may conclude that the *retention varies with the slowness of absorption*, and from 1 and 2 that *the absolute amount retained varies as the quantity injected;* whilst the *proportion retained is inverse to the quantity injected.* A more minute analysis also shows

that, while this is the general tendency, the proportion retained is less readily influenced by the injected quantity, than is the absolute amount retained, differences of 50% in the injection altering the proportion excreted but very little.

A relation can also be made out between the retention and the rapidity and duration of the excretion of albumen. Retention varies with the length of time required for excretion, but inversely to the quantity excreted in the first 24 hours.

Evidently the less the amount of albumen introduced and the slower its absorption and excretion, the more thoroughly is it retained. It may be assumed that the capacity of the organism for its utilization is limited, and these conditions would be the most favorable to it.

However, certain unknown factors also exist, as might be expected. Thus, in experiments XVI and XVIII the same amount was injected in the same manner: One animal retained 44%, the other 61%. But such instances are surprisingly few.

C. Nature of the Excreted Proteid.

The identity of the excreted with the injected proteid has been amply demonstrated for various substances (Munk and Lewandowsky). Stokvis showed by polarimetric methods that this holds true for egg-albumen. We thought it interesting to compare the *fractional coagulation temperatures* of the injected and excreted proteids and found the closest agreement. We often encountered some globulin in the urines; its quantity was ordinarily very small, and could be referred to the traces existing in egg-white. In certain cases a considerable quantity was excreted, but in these there was usually some other indication of nephritic changes.

D. Excretion of the different Constituents of Egg-Albumen.

The hypothesis has been repeatedly advanced that the precipitates which occur in solutions of egg-albumen at 57.5; 67; 72; 76; and 82° C. represent so many distinct chemical entities. Since we have in the kidney an instrument which is evidently capable of separating with great nicety very closely allied proteids, the thought suggested itself that the partial retention of egg-albumen was perhaps due to the complete retention of certain of these constituents, with

complete excretion of others. To solve this problem, we determined quantitatively the ratio of the precipitates at different temperatures in the injected solution and in the urine.

To secure strictly comparable results, both were largely diluted with water. To make the salt concentration the same in both the injected solution and the urine, the latter (previously boiled and filtered) was added to the albumen solution in the same quantity as was used for the estimation of the proteids in the urine itself. The acidity was also carefully brought to the same degree in both. The injection was always made intravenously.

Table VI gives a compilation of the ratios:

TABLE VI.

Ratio of Proteids Precipitating at Different Temperatures.

	Injection.		Urine.		Serum.	
Degrees Centigrade	68 : 70 : 73 : 77		68 : 70 : 73 : 77		68 : 70 : 73 : 77	
Experiment XXII..	36.4 : 15.2		56.6 : 11.1			
	51.6	48.4	67.7	38.3		
Experiment XXVI.	31.8 : 0	19.4 : 50.9	27.3 : 16.2	15.8 : 40.7		
	31.8	70.3	43.5	56.5	92.5	7.5
Experiment XXIX.	29.7 : 9.8	15.6 : 45.6		29.1 : 60.3	86.5 : 8	0 : 5.6
	39.5	61.2	10.6	89.4	94.3	5.6

The figures give the percentage of the total proteid, precipitated at the given temperature.

It is seen that in experiment XXIX, where there was no admixture of globulins, and where serum-proteids can therefore probably be excluded, the lower proteids (68° and 70°) are lessened, *i. e.*, they are retained to a greater extent, or else their coagulation temperature is raised.

Experiments XXII and XXVI, on the other hand, show a relative increase in the lower proteids; but since these contained a large amount of globulins there was an undoubted excretion of serum, and since the latter contains almost purely the lower members, the result is not surprising.

If we calculate the quantity of serum in these urines on the basis, that the serum-albumin amounts to one half of the globulin,—certainly

the smallest possible amount *—and subtract the serum proteids calculated on this basis from the total proteids of the urine, these experiments will also bear out the conclusions formulated from experiment XXIX, the ratios now standing:

	Solution injected. 70° : 77°	Urine. 70° : 77°
Experiment XXII.....	51.6 : 48.4%	34 : 61%
Experiment XXVI.....	31.8 : 70.3%	17.9 : 82.1%

As a converse of this, we investigated in experiment XXX the unabsorbed liquid remaining in the abdominal cavity of a guinea-pig which had died between 3 and 14 hours after an intraperitoneal injection.

	68°	70°	73°	77°
Albumen injected	37.6	25.8	12.	26. %
Peritoneal liquid	53.	13.	21.	12. %

There is a greatly increased proportion of the lower proteid. We did not investigate whether this was due to admixture of serum, or to slower absorption of the lower portion of the egg-albumen.

It having been proved by the above experiments that *the lower proteids are either retained in larger proportion or changed into those of higher coagulation temperature*, we tried whether the higher proteids would be converted into the lower. For this purpose we injected an egg-solution which had been kept at 73° C. for 10 minutes. The urine gave *no precipitate up to 73°*, but a copious precipitation occurred above this temperature. 1.673 grm. had been injected, 0.617 was recovered in one hour.

We consider that this fact lends support to the view that the precipitates occurring in egg-albumen at different temperatures really belong to different proteids; the lower being more completely retained by the body, or converted into proteids of higher coagulation temperature; whilst the higher are less completely retained, and cannot be converted into the lower.

Friedenthal and Lewandowsky (10) have shown that serum loses its toxicity by heating to beginning turbidity (55 to 60°). The above experiments, in showing that excretion occurs when the solu-

* Cloetta (9) finds in acute nephritis in rabbits produced by aloin that there are 2.37 to 3.71 times as much albumin as globulin in the urine.

tion has been heated to 73°, prove that the non-retention of egg-albumen cannot be due to its content of some such toxin.

In this connection we investigated whether the kidneys of *chicken* also recognized ovalbumin as a foreign proteid.

Since the animals frequently died during or shortly after intravenous injection, the intraperitoneal method was resorted to. The fæces were extracted with water, and this extract tested for proteids in the usual manner.

Exp. XXXI.—Rooster.—Injected solution containing 0.763 grm. egg-albumen. None recovered on two days following.

Exp. XXXVI.—Hen.—Injected 2.275 grm. egg-albumen in solution. On following day the fæces yielded no globulin, but albumen as follows:

68°	70°	73°	77°	total
none	0.1305	0.1230	0.1135	0.3670

The chicken therefore behaves towards the injection of egg-albumen precisely like mammals; the retention of the lower proteids is still more complete.

E. Duration of the Albuminuria.

In experiments in which no nephritis existed, the albumen disappeared from the urine in:

				Mean.
Dogs.	Intravenous injection	(4 cases),	$1\frac{1}{2}$ to $2\frac{1}{2}$ days.	2 days.
"	Hypodermic "	(2 "),	2 to $3\frac{1}{2}$ "	3 "
Rabbits.	" "	(1 "),	3 "	
"	Intraperitoneal "	(5 "),	2 to 3 "	$2\frac{1}{2}$ "

In 4 experiments on dogs in which nephritis developed (as shown by the excretion of a larger amount of proteid than that injected), the albuminuria lasted from 3 days to several weeks, *i. e.* as long as the animal was kept under observation. Two dogs which excreted non-coagulable proteid after hypodermic injection showed this from 4 to 11 days.

It appears from the above that albuminuria in typical experiments lasts from $1\frac{1}{2}$ to 3 days, according to the manner of introduction. The comparatively long duration after hypodermic and intraperitoneal injection may be referred in large part to slow absorption of the solution.

A large lump persisted for two days at the site of hypodermic injections in rabbits; and, in cases of intraperitoneal injection, proving fatal in three hours or more, much liquid with white flakes was found in the abdominal cavity, although no other sign of inflammation was perceptible.

R. Winternitz (38) found that, after the injection of sterile egg-albumen into the pleural cavity of dogs, the amount of liquid in the pleura had considerably increased beyond the injected quantity in six hours; in sixteen hours, in one dog, it had only fallen to one-half; in another dog there was a trifle more than the injected amount after seventeen hours. The pleural liquid in all three animals contained flakes of coagulated proteid.

The excessively long persistence of a slight albuminuria in a few cases need not necessarily be referred to the injection. Haack (20) claims that the dog's urine frequently contains some albumin. M. Kaufmann (21) quotes F. N. Schulz, that rabbits show a light degree of albuminuria from the most insignificant causes; and Kóssa (23) claims that an idiopathic albuminuria is so frequent that of the numerous rabbits bought in half a year for the physiological institute in Budapest, scarcely two or three were free from it. Others, obtained from a different source, developed it after being caged for a few weeks. The rabbits used by us for quantitative experiments were always free from any noticeable albuminuria.

Our results bear out the statements of other observers on the excretion of egg-albumen. Munk and Lewandowsky (8), after injections into the ear-vein of rabbits, found the albumen mostly excreted on the 2nd day, with perhaps a trace on the 3rd. O. Weiss (11) also found quick excretion after intravenous injection of serum into the rabbit; but on subcutaneous injections he sometimes saw it last for weeks.

Forster (6), using much larger quantities, found all excreted on the 3rd day. Lehmann (7) found the excretion almost complete in one day, except in one case in which there was nephritis.

F. Beginning of the Excretion of Albumen.

The excretion always began quite rapidly. In Experiment I, in which particular attention was directed to this point, traces were made out in seven minutes after injection; the quantity increased gradually, until in 22 minutes the urine became solid on heating.

TABLE VI.—PERCENTAGE OF THE INJECTED ALBUMEN EXCRETED ON SUCCESSIVE DAYS.

	Dogs. Intravenous.						Dog. Hypodermic.	Rabbit. Hypodermic.	Rabbit. Intraperitoneal.				
Experiment and amount injected...	XIX. 2.849	XVIII. 2.840	V. 2.248	XVI. 1.892	Forster. 73.3	IX. .0153	X. 4.1364	XXVIII A. 6.469	XXVIII B. 5.850	XXXVII A. 4.313	XXXVII A1. 3.493	XXXVII B. 4.313	XXXVII B1. 3.493
First 24 hours.....	17 hours. 33½%	18 hours. 38.4%	23 hours. 49.5%	38.1%	62%	48 hours. 13 times.	55 hours.	5.1	13.9	10.3	12.7	17.4	8.1
Second 24 hours.....	17 to 41 hrs. 21.6%	18 to 42 hrs. 14.5%	23 to 43 hrs. 13.9%	24 to 42 hrs. 0.9%	8.4%		32%	4.6	14.2	10.3	6.8	13.9	10.4
Third 24 hours	Dead.	42 to 67 hrs. 2.8%	43 to 64 hrs. 1.6%	None.	2 %		None.	5.1	5.3	1.0	3.0	0.8	7.3
Fourth 24 hours.....		Trace.	None.					Dead.	Dead.	0.5	None.	None.	None.

It is seen in Table VI that when the injection is *intravenous* (4 experiments), *about ¾ of the excretion takes place inside of the first 17 hours; the remainder in the next 15 hours, and only traces later.* Where hypodermic and intraperitoneal injections are made, the excretion is more nearly equal on 3 successive days, or may even be higher on the second day than the first. This may be explained by slow absorption.

As to the *quantity excreted in the first few hours*, this must be largely influenced by diuresis—an extremely variable factor.

In one experiment (XXXII) of intravenous injection in a rabbit, in which the animal received 1.663 grm., 37% of the total amount was excreted in one hour. This certainly shows that the process of excretion is a very rapid one.

H. Retention of other Proteids after Intravenous or Subcutaneous Injection.

The recent work of Munk and Lewandowsky (8) has demonstrated the practically complete retention of casein and nucleo-proteid, and the retention of 85% of gelatine. Schaefer and collaborators (22) have shown that considerable proportions of proteoses and similar substances are retained. The complete assimilation of serum-proteids—whether from the same or from foreign species—is almost universally accepted. The common statement is that *alkali-albumin* is also completely retained. We made some experiments on this point.

Exp. VI.—Animal died under anuria.

Exp. VII.—Intravenous, 0.028 grm. per kg. Animal excreted both native and alkali-albumin. Probably some nephritis.

Exp. VIII.—Hypodermic, 0.344 grm. per kg. Urine proteid-free.

Exp. XI.—Intravenous, 0.615 grm. per kg. Urine proteid-free.

Our results therefore agree with those of other observers. We further determined that actual conversion was necessary, for a simple solution of egg in 0.5% sodium carbonate caused the appearance of a large amount of native albumen in the urine (experiment IV).

The statement of the text-books is that *myosin* is not excreted. This appears to rest entirely on an experiment of Lehmann (7) made with frog's myosin. In six experiments we injected extracts of

human, rabbit's, dog's and chicken's muscle into rabbits, intravenously or into the peritoneum. In no case was there any excretion. *The myosin of foreign species is, therefore, also completely retained.*

IV. EFFECTS UPON METABOLISM.

From the experiments detailed in the preceding section, it results beyond doubt that a considerable proportion of the injected egg-albumen is not excreted in the urine. Does this leave the body by any other channel? If not, is it metabolized in the same manner as proteid entering the organism by the normal channel?

Semmola (12) states that the injection of egg-albumen causes the appearance of proteid in the bile. The original paper was not accessible, but the reference gave no data as to the quantity excreted by this channel.

In the last metabolism experiment (XXXVII B) it was noted that the freshly passed fæces on the day following the injection were rather slimy. 5 grm. were extracted with water, and yielded coagulable proteid corresponding to 0.028 grm. nitrogen for the 16 grm. of fæces passed in 24 hours. A glance at the nitrogen of the fæces will also show that there is a tendency to an increase in the percentage of nitrogen on the first or second day following the injection.

The quantity due to the injected proteid cannot, however, be calculated, since the normal variations in the amount of fæces are so great. Thus, Rabbit A, in 2 periods of 3 days following injection excreted 2.142 grm. N in fæces: on other 6 days 1.774; an *increase* of 0.366 in the periods after injection. Rabbit B, after injection 1.467; other days 1.734, a *decrease* of 0.267 for the periods after injection.

We judge from this that the amount leaving the organism unchanged by way of the fæces must be quite small. A large amount remains to be accounted for. Stokvis (5) states that some is contained in the saliva, but this amount must be very small.

Most observations on the effect of proteid injection upon metabolism have been made upon serum. Several of the older authors mention that this is excreted completely as urea, but we could not learn how minutely the observations were made. Forster (6) made observations

on serum and egg-albumen injections; Pflüger (13) shows that in these experiments more urea was excreted than corresponded to the amount injected. Of more recent workers, Arloing (14) finds an increased excretion of N and P_2O_5, but gives no data concerning the relation of the increase to the amount injected. Albertoni (15) finds that the injection of defibrinated blood into the peritoneal cavity of starving or insufficiently nourished dogs caused no increased N excretion, if the animals had previously been well nourished, but increased the nitrogen of the urine if the animals had not been in good condition.

For egg-albumen we can only find the quoted experiments of Forster and statements of Laborde (16) that hypodermic injection into starving rabbits causes an increase of N excretion above the amount injected; but he does not give numerical data in support of his statements.

We decided to investigate this problem, and to give attention also to the form in which the nitrogen is excreted. The analyses for this purpose were made according to the methods laid down in Section II, p. 223. We shall first give a brief account of the experiments and the conclusions to which they lead, and shall follow this with the tables giving the quantitative results from which the conclusions were drawn.

We began our experiments with a dog (experiment XL). Having investigated the changes in metabolism resulting from starvation, and having demonstrated that the hypodermic injection of colloid (gum arabic) was without effect upon metabolism, we introduced subcutaneously 0.125 grm. per kilo. of egg-albumen. Neither coagulable nor non-coagulable proteid appeared in the urine, nor could any alteration in other ingredients be determined with certainty. Precisely the same negative result was obtained by the injection of respectively 0.069 and 0.125 grm. per kilo. into the ear-vein of rabbits (experiment XXIV A and B). We omit these experiments from the tables.

It appeared, therefore, that a much larger quantity needed to be injected to yield conclusive results.

We made two observations on starving rabbits (experiment XXVIII), and two (experiment XXXVII), of two injections each, on rabbits having a free supply of oats, the amount of the latter being weighed each day, and the quantity of N in the food being calculated from this.

It was found impossible to maintain a nitrogen equilibrium. In the case of the starving animals there was a rapid starvation-increase which had to be allowed for. In the fed animals, the injection produced a marked loss of appetite, so that the nitrogen balance had to be calculated from the income and output.

The results show that a considerable part of the injected proteid leaves the body unchanged. Another, smaller, portion is excreted as non-coagulable proteid. The percentage of both was much smaller in the starving animals, but this was probably because less was injected. A certain amount was excreted by way of the fæces.

Taking the rabbits of experiment XXXVII. as showing the minimum retention we have:

Of the 1.249 grm. of N (as egg-albumen) injected into the peritoneum in two doses, 5 days apart,

	Rabbit A.	B.
excreted unchanged in urine,	22.3%	32.7%
" as non-coagulable proteid in urine,	18.7%	25.5%
" as coagulable proteid in fæces, about,	5. %	5. %
Total excreted as proteid,	46. %	63. %

The remaining 54 and 37% (0.675 and 0.463 grm. of nitrogen) must have been either retained or metabolized.

It is seen from the figures that the excretion of the total nitrogen is invariably increased, and generally beyond the amount injected, so that not only is none of the proteid retained, but its injection causes the animal to lose also body-nitrogen. On this account it is not possible to state in what time this excretion of the metabolized egg-albumen occurs.

As to the form in which this is finally excreted, it is seen that the ratio of total nitrogen (excluding that of the proteids) to urea and ammonia is not changed, so that we must assume that the metabolism ends in the normal products, *i. e.* mainly urea.

TABLE VII.—METABOLISM-RESULTS OF EXPERIMENT XXVIII.

Two Rabbits were used: A ♂ 2460 grm. on July 1; B ♀ 2310 grm. They had been kept on an unlimited diet of oats and water for 10 days previous to July 2, 1900, when oats were withheld. The observations were made at 1 P. M.

	Rabbit A.						Rabbit B.					
	July 6.	July 6 to 7.	7 to 8.	8 to 9.	9 to 10.	10 to 11.	July 6.	6 to 7.	7 to 8.	8 to 9.	9 to 10.	10 to 11.
Day of starvation	3d.	4th.	5th.	6th.	7th.	8th.	3d.	4th.	5th.	6th.	7th.	8th.
N in faeces		0	0	0	0	1.105		0	0	0	0	0.630
N in urine, total		1.663	1.890	2.667	3.328	1.747		1.393	2.844	2.468	3.259	2.053
" urea		1.313		2.233	3.166	1.663			2.664	1.900	2.849	1.035
" non-urea		0.350		0.434	0.162	0.084			0.180	0.568	0.410	0.118
" ammonia		0.011	0.010	0.030	0.031	0.037			0.010	0.004	0.019	0.041
" non-coagulable proteid (bromine)		0.151	0.110		0.137	0.113			0.158	0.180	0.129	0.075
" coagulable proteid				0.053	0.048	0.053				0.126	0.133	0.050
Per cent. of total N of urine in urea		79		84	95	95			93	77 *	88	94
Ammonia		0.7	0.5	1.1	1.0	2.			0.4	0.1	0.6	2
Non-coag. proteid		9.0	6.0		4.0	7.			6.	7.4	4.	4
Weight = % of original weight		87.4	84.6	83.3	78.5	65.0	88.7	87.2	82 3	79.4	71.9	56.5
N loss = % of weight loss (exclusive of coagulable proteid)	89.8	2.77	2.70	8.89	2.77	0.5		4.64	2.47	3.53	1.91	0.7
				Albumen = 1.035 N injected into perit. sinus at 3.15 P. M. of 8th. 9th, Large lump at site of injection.	10th, Lump almost gone.	Found dead at 8.15 A. M. of 11th.				Albumen = 0.936 N injected into peritoneum at 3.15 P. M. of 8th.		Found dead at 8.15 A. M.

* = 81% of total N exclusive of proteid.

TABLE VIII. METABOLISM OF RABBIT XXXVII A.

♀ 975 grm. Fed on oats. Observations made at 2 P. M.

July and August, 1900....	23 to 24.	24 to 25.	25 to 26.	26 to 27.[3]	27 to 28.	28 to 29.	29 to 30.	30 to 31.	July 31 to Aug. 1.	1 to 2.	2 to 3.	3 to 4.	Total from July 25 to August 4.
Total N consumed.....	1.017	0.261	1.396	0.863[1]	0.610	0.575	0.558	0.698	0.908[1]	0.872	0.767	0.715	8.961[1]
" excreted			1.356	1.312	1.038	0.363	0.504	0.564	0.835	0.597	0.651	0.496	7.579
Total N gained			+0.040	−0.449	−0.428	+0.212	+0.194	+0.134	+0.073	+0.275	+0.113	+0.219	+1.382
Weight gained (grm.).....	+30	−30	+40	+5.	−70	+25	−20	−10	+35	−5.	+15	+5.	+20
Difference in N = % of difference in body weight			0.1	Neg.	0.6	0.85	Neg.	Neg.	0.21	Neg.	0.8	1.0	6.9
N in faeces	0.260	0.341	0.600	0.388	0.681	0.095	0.112	0.315	0.394	0.250	0.333	0.146	
" urine			0.756	0.924	0.357	0.268	0.252	0.249	0.441	0.347	0.321	0.350	
N in urea.....			0.728	0.746	0.202	0.235	0.229	[0.210][2]	[0.270][2]	[0.230][2]	0.275	0.322	...
" non-urea.....			0.028	0.178	0.155	0.033	0.023	0.073	0.137	0.039	0.046	0.028	
" ammonia			0.035	0.040	0.007	0.009	0.012	0.025	0.022	0.028	0.008	0.003	
" non-coagulable proteid ($ZnSO_4$)				0.048	0.072	0.011	0.007	0.005	0.083	0.045	0.025	0.037	
" rest (theoretically alloxur, etc.)				0.049	0.006	0.006	0		Neg.		Neg.	Neg.	
" coagulable proteid.....				0.071	0.070	0.007	0.004		0.071	0.038	0.018		0.279
% of total nitrogen of urine (exclusive of proteids) in Urea			90	89	79	90	92		71.[3]	78.[2]	88.	92.	
Ammonia.....			4.6	1.2	2.5	3.4	4.8	10.	0.0	9.	2.7	0.9	
Non-coagulable proteid.....				5.5	25	4.2	2.8	2.	22.	12.	8.	11.	
				At 2.45 of 26th albumen = 0.680 N into peritoneum.					At 2.35 of 31st albumen = 0.359 N into peritoneum.				

[1] Including injected.

[2] Some urine lost, not over 10%; no correction applied.

[3] Direct analysis missed; estimated by difference.

TABLE IX.—METABOLISM OF RABBIT XXXVII B. ♀ 1185 GM.

Observations made at 2 P. M.

July and August, 1900....	23 to 24.	24 to 25.	25 to 26.	26 to 27.	27 to 28.	28 to 29.	29 to 30.	30 to 31.	July 31 to Aug. 1.	1 to 2.	2 to 3.	3 to 4.	Total from July 25 to Aug. 4.
Total N consumed	1.134	0.872	0.959	0.951[1]	0.523	0.872	1.221	0.959	0.646[1]	0.785	0.802	0.593	10.315[1]
" excreted	1.352	0.894	1.213	1.494	0.981	0.559	0.631	0.715	0.750	1.063	0.693	0.743	11.118
Total N gained	−0.218	−0.022	−0.254	−1.233	−0.458	+0.313	+0.590	+0.244	+0.603	−0.310	+0.109	−0.150	−0.803
Weight " (grm.)	+10	−10	0	0	−40	+25	+40	−15	+5	−30	+5	−15	−25
Difference in N = % of difference in body-weight	Neg.	0.22			1.1	1.2	1.5	Neg.	Neg.	1.	2.	1.	3.2
N in fæces	0.468	0.290	0.230	0.358	0.178	0.259	0.201	0.306	0.292	0.207	0.173	0.239	
" urine	0.884	0.604	0.983	1.136	0.803	0.300	0.430	0.409	0.458	0.886	0.520	0.504	
N in urea	0.805		0.917	0.941	0.566	0.260	0.396	0.380	[0.3383][3]	0.773	0.454	0.469	
" non-urea	0.079		0.066	0.195	0.237	0.040	0.034	0.019		0.113	0.066	0.035	
" ammonia	0.011	0.006	0.005	0.008	0.012	0.008	0.005	0.014	0.010	0.009	0.011	0.003	
" non-coagulable proteid ($ZnSO_4$)				0.067	0.125	0.011	0.010	0.011	0.053	0.097	0.022		
" rest (theoretically alloxur, etc.)				0	0.004	0.005	0.010	Neg.		Neg.	Neg.		
" coagulable proteid				0.120	0.096	0.006	0.009		0.056	0.072	0.050		0.409
% of total nitrogen of urine (exclusive of proteids) in													
Urea	91		93	84	80	87	93	97	84	95	97	93	
Ammonia	1.2	1	0.5	0.8	1.7	2.6	1.2	3.5	2.5	1.1	2.3	0.6	
Non-coagulable proteids				6.0	17.6	3.6	2.4	2.7	13.2	11.9	4.7		
				At 2.50 of 26th injected Albumen = 0.680 N into peritoneum.					At 2.40 of 31st injected 4.150 Albumen = 0.559 N into peritoneum.				

[1] Including injected.

[3] Direct analysis missed; estimated by difference.

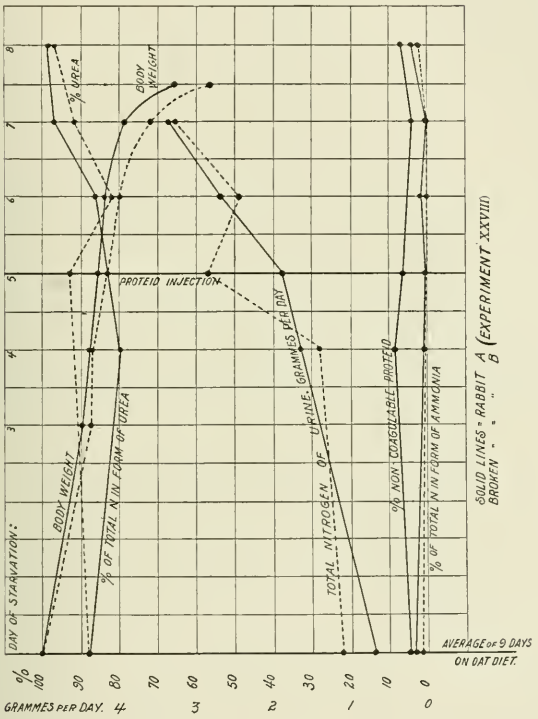
The presence of *non-coagulable proteid* as the result of hypodermic and intraperitoneal injections is an interesting fact. It was established by the ferrocyanide reaction after boiling, as also by the bromine and zinc-sulphate methods, so that it seems beyond doubt that these injections may cause the excretion of a substance having these properties. The amount was not sufficiently large to permit further characterization.

No such substance appeared in the case of any of the intravenous injections in which it was sought; nor did it occur in all cases of hypodermic injection. Its excretion sometimes persisted for a very long time—especially after repeated injections. In experiments XXXVII A and B, in which it was estimated quantitatively, the amount was proportional to the amount of coagulable proteid excreted (A: coagulable = 22.3% of the injected, non-coagulable = 18.7%. B: coagulable = 32.7%. non-coagulable 25.5%). There was no suppuration in these cases, so that this theory of its origin may be discarded. The fact that it did not occur on intravenous injection also disposes of the theory that it might result from the fever.

We believe that these albumoses represent a transition stage in the metabolism of the injected proteids. This would be in line with the observation of Krehl and Matthes (17) that deutero-albumoses are formed throughout the fever period, when there is also an increased proteid metabolism. The fact that it is not seen after intravenous injection can perhaps be explained by so much proteid being excreted unchanged that the organism may metabolize the small residue completely. This agrees with the fact that none is excreted after very small hypodermic injections. The fact of its long persistence in some cases favors the view that albumen injection may cause a long persisting alteration in proteid metabolism.

Experiment XXVIII gives good illustrations of the effect of *starvation on rabbits.* This subject has been investigated by Rubner (18), and more recently and thoroughly by Heymans (19), who draws his observations from 32 full grown rabbits, after withdrawal of both food and water. Our observations, which we present in the form of Curve I, show agreement with the latter observer as regards loss of weight (35 and 43.5%), the curve of loss of weight declining suddenly on the last day; and in the premortal rise of nitrogen

excretion. We wish to call particular attention to the slight change in the nitrogen during the first four days.



This agreement is remarkable in view of the fact that our animals were supplied freely with water. On the other hand, death occurred between the 7th and 8th day, whereas in Heymans' experiment it happened on the 6th to the 26th, averaging 15 to 16 days. That the early fatality might be connected with the proteid injection seems very improbable, since the loss of weight is so nearly the same as in Heymans'

observations. Some of Rubner's animals (18) also succumbed as early as the 4th day. Other instances are quoted by Kaufmann (21). We are inclined to refer it rather to the fact that our animals were young, and that their metabolism therefore was more active. The early occurrence of the starvation-rise in the nitrogen excretion, points in the same direction.

A striking feature in our cases is the fact that the proportion of urea to total nitrogen is notably increased in the last days of starvation.

Heymans also noted that it was higher during starvation than before, but since he made the urea determinations by a rather rough hypobromite test, he did not feel himself justified in laying stress upon the small difference. We believe that our methods are sufficiently trustworthy, and although the number of experiments is very small, yet they seem to justify the conclusion that the nitrogen metabolism in starvation tends to more complete oxidation than normally. This tends to support Voit's explanation of the cause of the premortal rise in nitrogen excretion, *i. e.*, that it is due to the body becoming relatively poor in fat; as against F. N. Schulz (24), who accounts for it by more proteid being thrown into the circulation by the extensive dying of cells; or another theory, which supposes that the cells become incapable of utilizing fat. With either of the last two theories, one would rather expect the oxidation of the proteids to be less complete.

V. PHYSIOLOGICAL AND OTHER PHENOMENA.

These will be considered in the following order:

A. Effects in Non-Fatal Cases.

1. Respiration and Circulation.
2. Temperature.
3. Diuresis.
4. Glycosuria and Hæmoglobinuria.
5. Histological Findings after Injection of Egg-Albumen.

B. Fatalities Occurring in the Experiments and Their Causes.

Anæsthetics.

Bacterial Infection.

Toxic Effects of Egg-Albumen.

Toxic Effects of Alkali-Albumin.

Myosin.

A. EFFECTS IN NON-FATAL CASES.

1. *Respiration and Circulation.*—During the earlier experiments on intravenous injections it was always noticed, incidentally, that the respiration increased. In two experiments (XXII and XXVI) special attention was given to this phenomena; tracings were also taken from the carotid artery and the venous pressure was measured by a water manometer connected with the central end of the femoral vein. In one animal the injection of egg-solution was preceded by an equal amount of normal saline, to allow of a comparison of the two. It was found that the effects were essentially the same in both cases. The respiration was deepened, the heart slowed and strengthened, both the arterial and venous pressures showing a small rise persisting through some 15 minutes. It may, therefore, be stated that intravenous injection of egg-albumen has no effects upon respiration and circulation beyond those of the liquid with which it is introduced.

This result differs considerably from that obtained with sera of foreign species (Arloing (14), Weiss (11), Friedenthal and Lewandowsky (10), &c.). Thus Arloing (14) describes after injection of horse serum into dogs a stimulation of the respiratory centre and a depression of the cardiac muscle and vascular tone. T. G. Brodie (25) finds that the intravenous injection of serum of any source into cats causes arrest of respiration, inhibition of the heart, and vasodilation. All these phenomena last for some time and are produced reflexly by stimulation of the pulmonary branch of the vagus. The active substance is an albumin, coagulating at 86° C., produced during the clotting of the blood. Repetition of the injections leads to immunity. These results are obtained only on the cat. Nothing of the kind was ever seen by us with egg-albumen or its modifications or with muscle on dogs or rabbits.

It must be remembered that the proteid content of our solution was only $\frac{1}{4}$ to $\frac{1}{2}$ as strong as that of serum. Stronger solutions are impracticable, since a proteid content of 10 to 15% causes speedy death from embolism, preceded by dyspnœa, then respiratory paralysis and lastly cardiac stand-still. Two deaths from this were recorded (experiments XVII and XLI). Another death (experiment XIV) from what was probably embolism from injected air occurred in four hours after injection, with symptoms of progressive medullary paralysis.

2. *Temperature.*—The statement is found in text-books (Lazarus-Barlow, General Pathology) that injections of proteids—particularly

albumoses, but also others—cause a rise of temperature. Quincke (26) notes this for the intravenous injection of defibrinated blood. Injection of other substances may also cause hyperpyrexia. Thus E. Haack (27) noted it after the subcutaneous injection of silver nitrate and tincture of iodine; these substances, however, cause simultaneously an albumosuria. Combemale and Mouton (28) found that the hypodermic injection of 20 cc. of normal salt solution produced a rise of temperature fairly regularly in tuberculous patients, but not in healthy individuals, nor in those suffering from other diseases. For animals, however, Thompson (29) has shown that the intravenous injection of small quantities of normal saline caused a rise of temperature to 2° C. Bose and Vedel (30) have seen the same effects from larger injections.

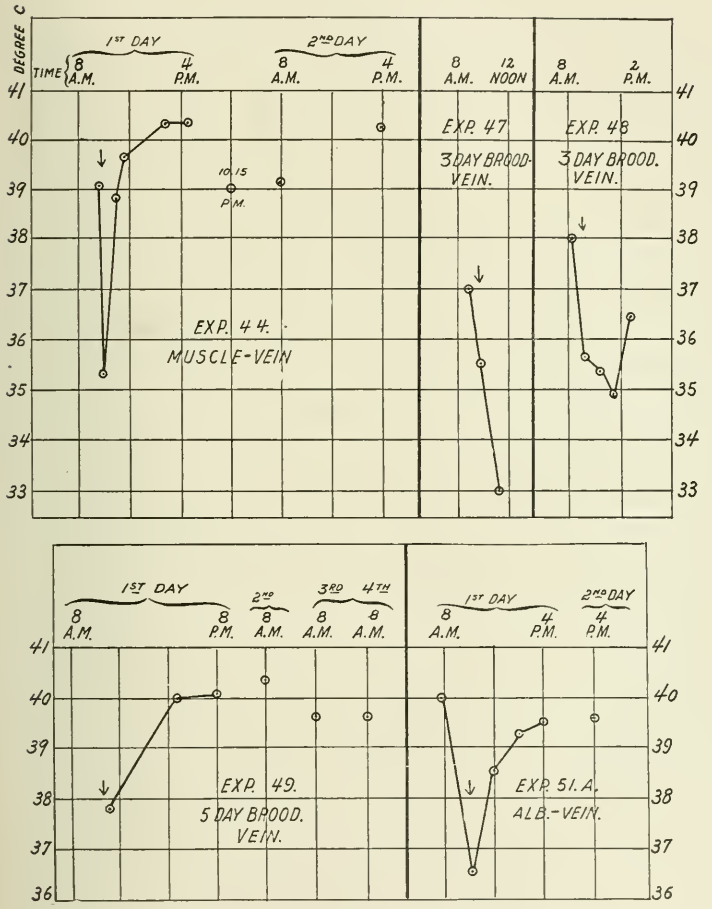
In a number of our experiments on rabbits we directed particular attention to the effects of various injections upon the temperature. A self-registering thermometer was inserted for a depth of 7 cm. into the rectum, and left in position until no further rise occurred. The results are represented in Curves II on Plates XVI-XIX. The arrow indicates the time of injection. Urethane was given in all the cases of intravenous injection

It will be seen that all the proteids which were tried (albumen, derived albumen and myosin) produce a very distinct rise of temperature. This occurs whether they are injected subcutaneously, or into the peritoneum, the ear-vein, or the jugular vein. In the last, the rise is often masked by the fall of temperature due to the anæsthetic.

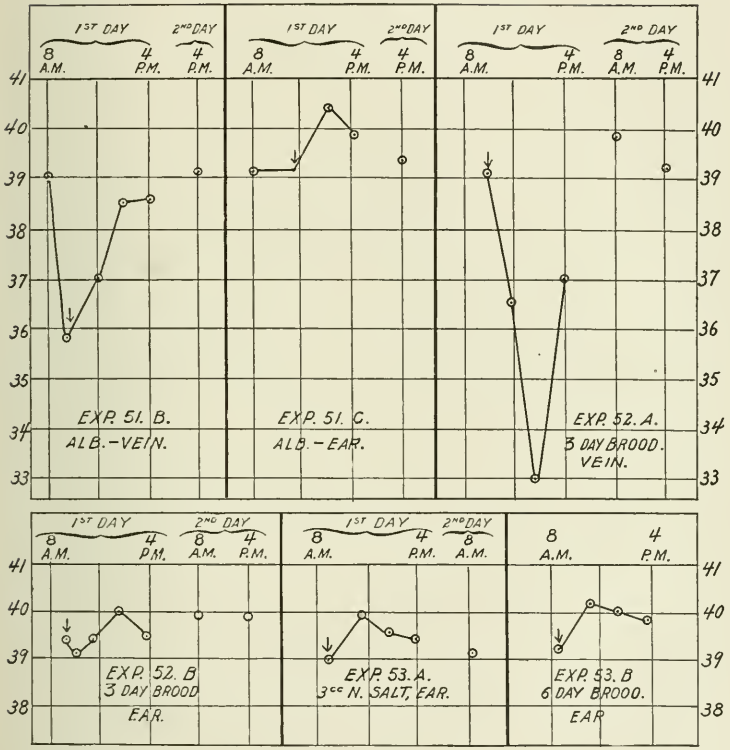
When the amount of the latter was very large, and especially when it was fatal, it prevented the rise entirely. Urethane alone, 0.5 grm. per kilo. by rectum, lowered the temperature 2.3° C. The existence of the bacterial infection, presently to be described, had no influence on the fever, either as to its height or duration. All the proteids had about the same effect.

The rise begins in about an hour after the injection of the solution, and reaches its maximum in from 6 to 24 hours (usually 6 to 8 hours). It then falls very rapidly, but in rare cases may persist for 2 or 3 days (Experiment 62, Plate XIX). A recurrence of the fever is

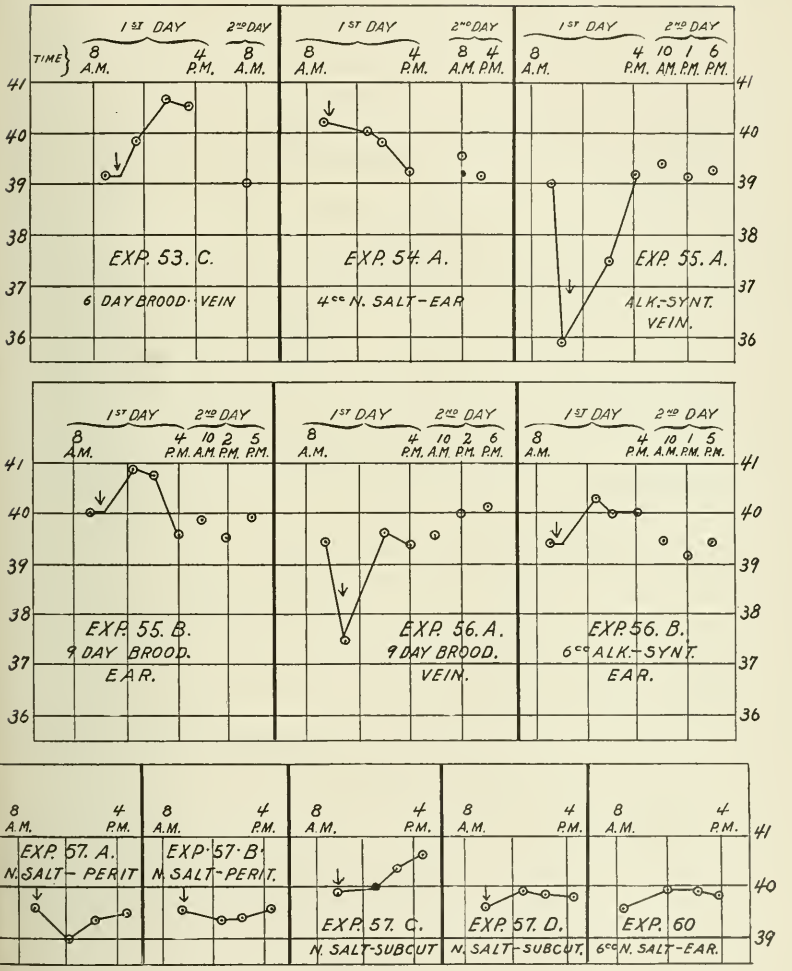
CURVES II.


DEGREE C
TIME
1ST DAY
2ND DAY
8 A.M.
4 P.M.
8 A.M.
4 P.M.
8 A.M.
12 NOON
8 A.M.
2 P.M.
41
40
39
38
37
36
35
34
33
10.15 P.M.
EXP. 44.
MUSCLE-VEIN
EXP. 47
3 DAY BROOD-VEIN.
EXP. 48
3 DAY BROOD. VEIN.
1ST DAY
2ND
3RD
4TH
8 A.M.
8 P.M.
8 A.M.
8 A.M.
8 A.M.
EXP. 49.
5 DAY BROOD.
VEIN.
1ST DAY
2ND DAY
8 A.M.
4 P.M.
4 P.M.
EXP. 51. A.
ALB.-VEIN.

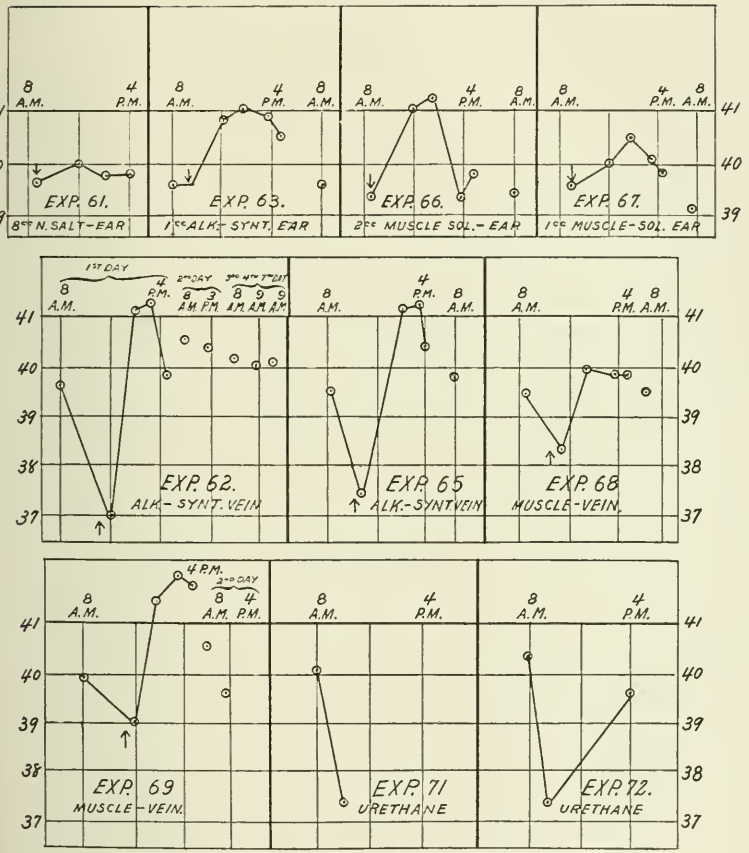
CURVES II—Continued.



CURVES II—Continued.



CURVES II—Continued.



sometimes seen on subsequent days, particularly in the afternoon. The rise is usually a trifle over 1°, but may be as much as 2° C. It seems to be almost independent of the quantity injected; at least, it may be produced by extremely small quantities (as in experiment 66 by 15 mg. of the proteid of rabbit's muscle, per kilo, of injected rabbit).

Normal salt solution alone, when injected into the ear, may cause some rise of temperature, but smaller than that following the use of the proteids (the mean being 0.35° C.). Subcutaneous injection may also cause a rise, but this is still smaller. In some cases the injection of normal salt solution, by way of the ear, skin or peritoneum, causes a slight fall of temperature (from reflex dilatation of the vessels?).

3. *Diuresis*—The injection of egg-albumen tends to cause a diuresis. In certain animals an anuria persists for many hours, but this may be seen without albumen injection, and even after the injection of a third of the body-weight of normal saline. The curve of the diuresis, as recorded in two cases, differs very greatly from that produced by the injection of normal salt solution (Sollmann 31).

With the latter the flow of urine reaches its maximum in ½ hour and has returned to a relatively small figure in 1½ hours. With albumen injection, on the other hand, there is a small primary rise, reaching its maximum in 15 to 20 minutes, and then a fall to 50 minutes. This curve is probably attributable to the salt solution which carries the proteid, since it is practically identical with that produced by salt solution alone.

About 50 minutes after the injection, however, a second diuresis sets in, reaching its maximum in about 2 hours, when it is 3 to 5 times greater than the primary rise. This must be due to a specific irritation of the kidneys by the albumen. We did not investigate whether this action is exerted upon the vessels or cells. The accompanying Curve III, from experiment I, gives a typical illustration of this phenomenon (Plate XX).

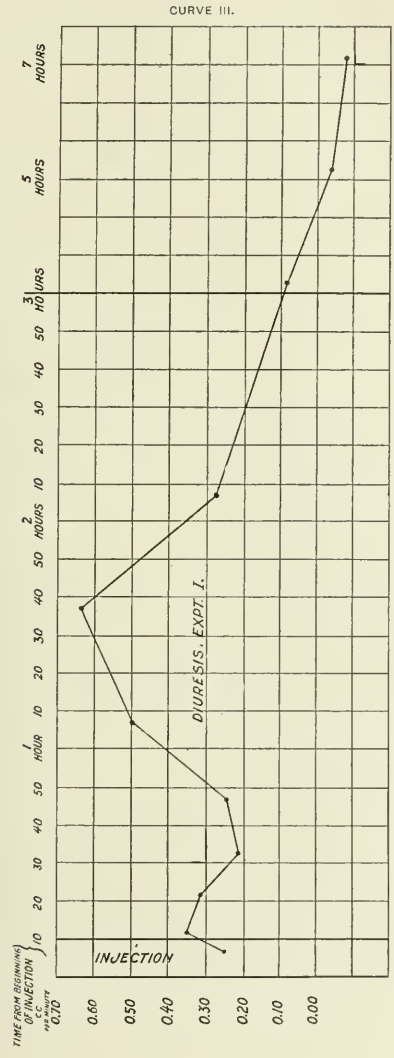
4. *Glycosuria.*—This never occurred on hypodermic or intraperitoneal injection. It was noted in the case of dogs, after intravenous injection, in 5 out of 18 animals. The quantity was deter-

mined in but two experiments (I and III). In the latter it reached 0.32 grm. per kilo. The excretion was completed inside of the first 24 hours, except in one experiment (XXI) in which it appeared on the second day after injection, the urine being free from it on the first. In this animal, morphine alone caused an abundant glycosuria.

Evidently the excretion of sugar must be referred to the anæsthetic and to the morphine. Injection of the proteids does not cause glycosuria.

Hæmoglobinuria.—This was observed in only one case (experiment XII) and could not be attributed to the injection of albumen. The urine remained normal for 7 days after a second hypodermic injection, when it suddenly showed oxyhæmoglobin and methæmoglobin, a strong content of albumin, but no casts. On the following day these had disappeared, but the urine now contained bile pigment. The animal was sacrificed on this day. The autopsy was negative, and microscopic examination of the kidneys did not reveal anything abnormal.

In one experiment on a rabbit (No. 48) the bladder at autopsy was found distended with a reddish urine, giving the oxyhæmoglobin spectrum. Microscopic examination showed the abundant sediment to consist of numerous red blood-cells and a large quantity of granular casts. These appearances were not present in any other experiment of the entire series. The animal, which had received some albumen-solution intravenously, had died 3½ hours after the injection, evidently from the effects of an overdose of the anæsthetic (urethane, 1.18 grm. per kilo.; temperature fell to 35° C.). The peritoneum contained a large quantity of free blood, which clotted on removal. A rupture of the liver was noted, but it could not be decided whether this was inflicted at the autopsy (the liver tissue being extremely friable); or whether it had occurred earlier, perhaps during the injection. The microscopic examination showed considerable degeneration of the renal and hepatic epithelium. The last was certainly due to the urethane. The urine, which was of neutral reaction, showed a large quantity of coagulable proteid, but no sugar. This fact shows that the albumen was not due to the hæmorrhage, but that the excretion of the injected albumen was well advanced at the time of death. The marked nephritis, shown by


CURVE III.
DIURESIS. EXPT. I.
INJECTION
TIME FROM BEGINNING OF INJECTION
1 C.C. = 0.70 GRAMS
0.60
0.50
0.40
0.30
0.20
0.10
0.00
10
20
30
40
50
1 HOUR
10
20
30
40
50
2 HOURS
10
20
30
40
50
60
3 HOURS
5 HOURS
7 HOURS

the casts and by the histological appearances, can not have been due to the injection. Nor do we think that it could have been caused by the urethane, and we must leave it unexplained.

5. *Histological Findings After Injection of Egg-Albumen.*

Previous experimenters have found that the injection of proteids causes slight, but distinct, nephritic changes. Semmola (12), Laborde (16) and Seigert report parenchymatous nephritis after injection of egg-albumen. Laborde also mentions necrotic foci in the liver; Semmola reports retinal changes. O. Weiss (11), injecting foreign serum into dogs, finds only hyperæmia of the renal cortex. A. Pettit (32) remarks that the injection of eel serum causes in rabbits a rapidly progressing degeneration of renal epithelium, noticeable even when the animal survives only a few minutes.

We were able through the kind assistance of Professor Wm. T. Howard, Jr., to subject the greater part of our material to histological examination. The methods employed have been described in Section II (p. 218). The animals were killed at various times, but usually several days after the injection.

The *kidneys* of nearly all the animals examined showed a greater or less degree of congestion of both cortex and medulla. Those of the dogs, after injections of plain albumen, presented no noticeable degeneration. In the rabbits on the other hand, the epithelium of the convoluted tubules was almost always cloudy and swollen, sometimes even hyaline or vacuolated. Granules of iron-free pigment were seen in the epithelial cells in many cases. Hyaline casts, resulting from coagulation of the albuminous urine, were often seen in the tubules. These changes, except the last, are such as we often saw in animals which had received no injections. It is quite possible, though by no menas certain, that they are more intense after the injection of the proteids.

In experiment 48 alone, described on page 244, was the degeneration at all extensive. This was also the only case in which casts were at all numerous in the urine. The cause of the peculiarities of this experiment must stand unexplained.

We may say, then, that the injection of egg-proteids does not lead to any conspicuous nephritic lesions. This holds true for dogs, rabbits and guinea-pigs. A slight degree of cellular degeneration cannot be excluded. The constant appearance of congestion points to some inflammatory action. Certain experiments give further evidence

of nephritic changes through a long continued albuminuria, through an acute globulinuria, etc.; but these changes are evidently not of such a degree as to give rise to marked degenerative lesions.

The intravenous injection of an extract of rabbit's *muscle* into two rabbits (experiments 68 and 69), showed very marked and extensive degeneration of the renal epithelium. The amount of urethane was very small in these cases (0.33 to 0.35 grm. per kilo.), so that the changes could not be attributed to it. Moreover, the other organs were normal. The degeneration was much more marked in the animal killed three days after injection, than in the one killed after 10 days. The kidneys showed no alterations in a case (experiment XLIV) of intravenous injection of dog's muscle and in experiment XXXIII of intravenous injection of human muscle into rabbits; nor in 2 rabbits (experiment XXXVIII), into whose peritoneum chicken's muscle was injected; nor in two others (experiment XXXIX), which received dog's muscle in the same manner. The degeneration of the renal epithelium, as noted in experiments 68 and 69, was therefore peculiar to this particular extract, and is not a common sequence of injections of muscle extracts.

The absence of conspicuous pathological lesions holds true still more for *other organs.* The spleen, intestines, pancreas and adrenals showed at most some congestion. The liver epithelium was very often greatly degenerated in rabbits which had received large doses of urethane; but this was plainly referable to the latter. Parasitic changes were often seen, and coccidium oviforme was very often found in the bile-ducts. In experiment 65 numbers of amœba-like parasites were seen in one kidney.

The absence of conspicuous degenerations is of interest not merely from its connection with the effects of proteid injection, but also because it bears on the much discussed question, whether uncomplicated hyperpyrexia causes cellular degeneration. We have not seen any such after elevations of temperature of one or two degrees lasting for two days. This tends to support Naunyn's view, corroborated lately by E. v. Czyhlarz (33), who saw no degenerations after cerebral puncture in ten rabbits.

Some attention was given to the distribution of pigment. Both hæmofuscin (iron free) and hæmosiderin (iron-containing) were found, in variable amount, in all cases. The latter was exclusively in the spleen. The former in the liver epithelium, in the spleen, both free and in cells, and often in the renal epithelium.

B. FATALITIES OCCURRING IN THE COURSE OF THE EXPERIMENTS AND THEIR CAUSES.

Many of our animals succumbed in the course of the experiments. The greater number of these deaths could be referrd to well defined causes. Amongst these are embolism through two great concentration of the solution or from air; overdoses of the anæsthetic; bacterial infection; excessive handling or long exposure to unhygienic conditions, etc. A certain number pointed to a specific toxicity of the proteid. It seems to us of interest to give an illustrative description of the course and of the gross and microscopic changes in the principal fatal cases.

The phenomena of embolism are dealt with on page 241.

Deaths from the Anæsthetic.—Only one late death was observed in a dog (experiment XIX) which had received morphine and ether.

Experiment XIX.—This animal was very listless on the day following the operation; later it lay almost motionless in a comatose condition, and was found dead on the following morning. Autopsy negative.

The majority of deaths occurred in rabbits, after chloretone or urethane. The fatal dose for *chloretone* by stomach catheter, lies slightly above 16 cc. of a saturated solution per kilo. The symptoms were very similar to those produced by urethane. The degeneration of the liver cells was absent, nor were there any other histological lesions. There was, however, in all cases a marked capillary congestion of the abdominal organs.

The fatal dose of *urethane* given in 5% solution per rectum, lies between 0.75 to 1.0 grm. per kilo. But doses of 0.6 grm. cause very severe degeneration of the hepatic cells. Franz Müller (37), found that 1 grm. of urethane per kg. killed one rabbit out of three (in 2 days). When given by mouth the fatal dose of these anæthetics lies somewhat higher than by the rectum.

The following experiment forms a typical illustration:

Experiment XXXIII.—July 18, 1900. Rabbit ♂, 1790 grm., was anæsthetized with 1.5 grm. urethane, and ether; the jugular vein was exposed under aseptic precautions, and 125 cc. of a solution of human muscle in normal salt solution (= 0.486 grm. proteid) were injected.

The animal was partly conscious at the end of the operation (2.30 P. M.) and appeared in good condition. The wound was sutured and the rabbit placed in its cage. At 5.30 it was found lying on its side, very much worse. There had been, and was at this time, a profuse diarrhœa and

involuntary passages of a small quantity of urine. The heart was very fast; respiration 140; temperature 36.1° (normal rabbit = 40° C.); pupils of medium size, ear-vessels normal; reflexes slow. At 7 p. m. the respiration was 140; temperature 35.1°, heart fast, reflexes lost except corneal: pupil rather small. Heated bricks were placed about the animal at 7.15. At 7.25 the respiration became slowed to 12 and was coughing and spasmodic; temperature 34.4°. The animal died at 7.28, the respiration ceasing before the heart.

The *autopsy*, made immediately after death, showed a congestion of all abdominal organs. The small intestine was contracted and contained mucous fluid. The bladder was empty. The lungs were pink; the heart was not distended. The liver and kidneys presented a peculiar waxy appearance.

Histological Examination: (This refers to all the experiments in which urethane was used in a dose of 0.6 grm. per kg. or larger. With a dose of 0.35 grm. per kg. there were no noticeable lesions).

The principal changes are found in the *liver*. There is an intense diffuse degeneration of the hepatic epithelium, more marked in foci. The cells stain unevenly, are very granular, and present numerous vacuoles. Fatty degeneration is also sometimes seen. The nuclei are fairly normal. The perilobular tissue is normal. After large doses a congestion of the central vein and capillaries is also seen. The process of degeneration must be a very rapid one, for it is fully developed in animals which died 1½ hours after the injection (experiment XLVII). In the kidneys, some degree of congestion is common. The epithelium is always somewhat granular, but not more so than may be encountered in normal animals. The spleen is always congested. The other organs—intestine, medulla oblongata, pancreas, cardiac muscle, adrenals, are normal. The distribution of pigments shows nothing unusual.

Deaths from Bacterial Infection.—During May, 1901, an epizootic made its appearance amongst the guinea-pigs and rabbits at the laboratory, and carried off a great number of the former. The mortality was not so great with the rabbits, perhaps because they were not kept sufficiently long. With these animals, the disease was not suspected until the sections were examined. The disease occurred in animals which had not been handled, and was therefore not due to the injection.

The *microorganisms* as seen in the tissues were for the most part rather short pleomorphic bacilli, but larger numbers of cocci were often seen, especially in the lungs. Agar cultures were made from the guinea-pigs, but could not be examined for several weeks. They then showed a number of organisms, of which only B. coli communis could be identified. We are indebted to Mr. H. J. Gertenberger for these cultures.

The rabbits, even when intensely infected, showed but slight symptoms

during life. The guinea-pigs became listless on the last day, did not eat, lay on their side, and had a gasping respiration. The *autopsy* itself did not show any conspicuous changes. The numerous cases which were examined permit us to pursue the histological lesions through the successive stages of the infection. The bacteria were seen in all the tissues. The kidney showed some change in the epithelium of the convoluted tubules; the cells becoming somewhat swollen and cloudy or hyaline. There were no casts. The connective tissue was often fairly abundant. With more intense infection, the cell degeneration became very conspicuous. The connective tissue showed considerable round-celled infiltration. Gas-spaces were discernible. The liver cells showed at first a moderate, diffuse, granulation. In the perilobular tissue there was frequently more or less pronounced infiltration with young connective-tissue cells. The degeneration of the epithelium became very profound with the progress of the infection, being also somewhat focal. There were severe congestions and hæmorrhagic areas, the latter containing hæmosiderin. The spleen was congested, but otherwise normal even in the severe grades. The cardiac muscle was very profoundly affected, containing a mass of bacteria, and its structure having largely disappeared. The lungs were congested; the alveoli, bronchi, and blood contained many bacteria. The intestine, adrenals and pancreas were normal.

There was some hyperleucocytosis, and an increase of eosinophiles, especially in the spleen. Hæmosiderin and hæmofuscin were unusually abundant in the more marked grades of infection, especially the hæmosiderin in the spleen.

Toxic Effects of Egg-Albumen.—The following very striking instance of death following the intravenous injection of egg-albumen, which occurred rather early in the course of the research, directed our attention to the possibility of a toxic action of proteids which are usually considered harmless, and led us to an extensive experimental investigation of this subject.

Experiment XVIII.—This dog was but lightly anæsthetized during the operation. On the day following it was lively, ran about, and seemed perfectly well. It remained so during three days, and even at 8 A. M. on the 4th day nothing abnormal was noticed. The animal was not observed again until 1 P. M. of the 4th day, when she was found in extreme clonic spasms and almost insensible. Restorative measures were resorted to, but failed, death occurring at 1.30 P. M. The autopsy and histological study were both negative. The wound had suppurated a little.

This case is particularly interesting, being the only one in which an animal died rather suddenly after several intervening days of perfect good health. It seemed to us worth while to attempt to reproduce the condition, to enable us to study it more carefully.

As we had seen this course but once in more than 20 animals which we had injected, we searched for methods of increasing any existing toxicity of the egg-albumen. For this purpose we first tried the effect of repeating the injections on animals which are usually very sensitive to toxic agents.

We chose 6 guinea-pigs for the purpose, injecting them always in pairs, to exclude other accidental causes so far as possible. The injections were sometimes made under the skin, sometimes into the peritoneum. We were always successful in obtaining a fatal result, both animals of a pair always dying at the same time. Death usually occurred during the night succeeding the last injection, so that the final symptoms could not be observed. The guinea-pigs were always somewhat depressed after each injection, but nothing else could be noted. The autopsy showed nothing abnormal. There was no sign of peritonitis. However, the number of injections required, as well as the amount of proteid, were so large, that it seemed unjustifiable to refer the death to them. We were forced to conclude that the injection of fresh egg-albumen was in no way toxic.

Mariani (189?) has shown that starving rabbits treated with hypodermic injections of egg-albumen die before the control animals, but this might be referred to the increased nitrogen metabolism produced by such injections.

We may state at this place that the injections ordinarily produced few or no symptoms in either dogs or rabbits, except that the animals tended to become emaciated while preserving a ravenous appetite.

The phenomena of experiment XVIII must therefore have been due to the presence of some extraneous toxin in the egg-albumen. It remained to determine whether this was due to infection of the egg-solution, or whether such a toxin could be developed in the unspoiled egg itself. The eggs used for this experiment were purchased in July, in the open market, and were of unknown age. Whilst the eggs were not visibly spoiled, the conditions were distinctly favorable to fermentation. The cholin, etc., of the yolk would form a favorable starting point for such ptomains. To decide this question, we took some freshly laid eggs and placed them under conditions which would be most conducive to such change, *i. e.* in a thermostat

at 40° C. Here they were kept for 3, 5, 6 and 9 days. In the last case, the yolk could not be separated, and had to be included in the solution. Animals injected with solutions made from these brooded eggs behave precisely as if injected with normal egg-albumen. Only one case ended fatally, and this five days after the injection. The histological examination showed thrombosis of veins in the liver, perhaps quite sufficient to cause death; but we doubt whether this result could have been due to the injection.

We conclude that pure egg-albumen is not at all toxic, nor does it develop a toxicity by prolonged sojourn at brooding temperature.

This leaves bacterial infection as the explanation of experiment XVIII. Bacteriological examination was unfortunately neglected, but the tissues were certainly not conspicuously invaded by any micro-organism. The symptoms resembled superficially those of traumatic tetanus; since the wound was open, infection by this channel could have occurred quite easily. However, the sudden outbreak, the general distribution of the convulsions and rapid fatality speak very strongly against the existence of bacterial tetanus. The absence of any such effects in other animals operated upon, and kept under precisely the same conditions, makes it unlikely that the infection occurred by way of the wound. The toxic agent must have been introduced with the injected solution.

(Fiquet (34), 1899, claims that even albumoses and peptones are not toxic on intravenous injection, when prepared sufficiently pure, and that they then have no influence on the clotting of blood. He refers the ordinary action to admixture of toxalbumins and ptomains).

Toxic Effects of Alkali-Albumin.—Only one fatal case occurred in six intravenous injections of alkaline egg-syntonin in dogs, and two in rabbits. In this fatal case the alkalinity of the solution was unusually high, and the amount large (35 cc. per kilo. of a solution containing 0.376% of free NaOH). The same symptoms and lesions could be elicited in another animal by the injection of pure $\frac{N}{10}$ NaOH solution, so that they are not dependent upon the proteid. We reserve their detailed description for another paper.

Briefly, the animals died in about 6½ hours, in coma and with convulsions. The autopsy revealed intense congestion of the abdominal

organs, particularly of the large intestine, with bloody effusions into the lumen of the alimentary canal and into the peritoneum.

Histologically, the main lesions were a necrosis of the intestinal villi and an acute interstitial nephritis, in every way similar to that described for the human subject by Councilman (35) and Howard (36). Hæmorrhagic areas were found in various situations. There was a profound hæmochromatosis, consisting mainly in the deposition of iron-free pigment granules in the intestinal mucosa, the liver, the blood, the lymph channels, and in the renal epithelium.

Other animals, which had received less alkali, showed much less marked changes in the same direction, or were free from lesions. Alkali-albumin, therefore, does not differ in its histological results from native albumen.

Muscle Extracts.—These did not possess any specific toxicity; the histological alterations corresponded to those seen after injection of egg-albumen.

VI. CONCLUSIONS.

The conclusions which we derive from our observations are as follows:

1. The excretion of injected egg-albumen as such is in no case complete. The quantity retained varies from 23 to 100%.

2. The amount retained varies:

 a) directly with the slowness of absorption. This is determined by the manner of administration.

 b) directly with the time during which the proteid remains in the body; and therefore inversely to the rapidity of excretion.

 c) inversely to the quantity injected; this has however much less effect than (a) or (b).

 d) with individual peculiarities; but these are not very conspicuous.

3. The excreted proteid coagulates at the same temperatures as the injected albumen.

4. Injection of egg-albumen does not cause the appearance of globulins in the urine.

5. The proportion of proteid coagulating at lower temperatures is less in the urine than in the injected solution. When a solution has been heated to 73° before injection, the urine also does not coagulate below this temperature.

6. Egg-albumen injected into the hen is excreted as with mammals.

7. The albuminuria lasts in typical cases from 1½ to 3 days, according to the manner of administration.

The excretion begins very shortly (7 minutes) after injection. 37 per cent of the total proteid injected may be excreted in an hour. About three-fourths of the total excretion takes place within the first 17 hours; the excretion is almost completed in the next 15 hours, only traces being excreted thereafter. With hypodermic injection the amount is more nearly equal on 2 or 3 successive days, since the absorption may extend over 2 days.

8. Alkali-albumin, as well as muscle-proteids (from foreign species) are completely retained. An unconverted mixture of egg-albumen and sodium carbonate behaves like egg-albumen.

9. A small amount of proteid (less than 5%) is excreted unchanged by the fæces.

10. A variable proportion is excreted as non-coagulable proteid. The quantity of this is proportional to that of the coagulable proteid of the urine.

11. The rest undergoes complete metabolism to urea.

12. The total nitrogen excretion is increased beyond the amount of nitrogen introduced as albumen.

13. Starvation appears to cause an increase in the ratio of the urea to the total nitrogen of the urine.

14. The effects of intravenous injection of egg-albumen on circulation and respiration do not differ from those of an equivalent injection of the solvent. Albumen causes, however, a specific diuresis, beginning 50 minutes after the intravenous injection, and reaching its maximum in about 2 hours. It causes neither glycosuria nor hæmoglobinuria.

15. The injection of egg-albumen, alkaline egg-syntonin, or muscle extracts, causes in rabbits a rise of temperature of 1 to 2° C.

This begins in about an hour, usually reaches its maximum in from 6 to 8 hours, and then falls rapidly. It may in rare cases persist for several days. It is indifferent qualitatively whether the injection is made by the jugular or the ear-vein, hypodermically, or into the peritoneum. Even extremely small quantities injected into the ear-vein cause this rise. The fever does not cause histological alterations in any organ examined. The injection of normal salt solution may cause a rise, but this is much smaller.

16. The injection of egg-albumen causes but very slight histological changes. The kidneys are usually congested, especially in the cortex. The cells may be slightly cloudy. A slight degree of nephritis may occur, but this is not of such degree as to effect permanent lesions. The injection of muscle extracts may give rise to a more pronounced parenchymatous nephritis.

17. Urethane is fatal to rabbits in doses of 0.75 to 1.0 grm. per kilo. The symptoms consist mainly in a very marked fall of temperature, and in medullary paralysis. 0.5 grm. per kilo. lowers the temperature 2.3° C. Doses as small as 0.6 grm. per kilo cause very marked histological changes, consisting mainly in extensive granular and vacuolar degenerations of the hepatic epithelium, which are so acute as to be fully developed when death occurs in 1½ hours after injection. Doses of 0.35 grm. per kilo. do not produce this change. Chloretone did not cause the degeneration, but is followed by congestion of the abdominal viscera.

18. Native egg-albumen, injected into the femoral vein of a dog, was followed in one case by a fatal ending with convulsions and coma, after several intervening cases of good health. Further experiments demonstrated that there is no toxicity inherent in fresh egg-albumen, nor can it be developed by brooding the eggs in the shell. The cause of the above fatal issue must therefore be sought in some extraneous toxic agent which contaminated the solution. Muscle-extracts were also devoid of toxicity. Alkali-albumin produces no changes beyond those which may be attributed to the free alkali contained therein.

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A CONTRIBUTION TO OUR KNOWLEDGE OF THE ACTION OF SAPONIN ON THE BLOOD CORPUSCLES AND PUS CORPUSCLES.

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I have shown in previous communications[1] that saponin produces a marked increase in the electrical conductivity of blood. The increase is quite as great in blood which has been fixed by formaldehyde as in fresh blood. It is, therefore, not dependent on the escape of the hæmoglobin from the corpuscles which saponin causes in unhardened blood, but not, of course, in blood treated with formaldehyde. I have demonstrated that no such effect is produced by saponin on blood-serum in the absence of the corpuscles. Its action on the conductivity of the blood may be explained in two ways: (1) It may increase the total conductance of the serum, (a) by causing the passage of electrolytes into it from the corpuscles without increasing its relative volume, or (b) by bringing about an increase in its volume, due to the passage of water out of the corpuscles and a consequent increase in the amount of dissociation of the electrolytes of the serum, or (c) by causing both water and electrolytes to pass out of the corpuscles.

(2) The saponin may increase the conductivity of the corpuscles without affecting that of the serum.

In the case of formaldehyde-hardened blood, where the action of saponin on the permeability of the corpuscles for electrolytes is most easily studied, since it is not complicated by the passage of the blood-pigment into the serum, it is not difficult to prove, by measuring the conductivity of the serum and sediment, respectively, after centrifugalization, that it is the conductivity of the corpuscles which is increased. If there is any increase in the conductivity of the serum,

[1] *Journ. of Physiology*, 1899, xxiv, p. 211, and 1901, xxvi, p. 470.

(a slight increase is perhaps indicated in some of the observations), it is small in comparison with the effect on the corpuscles. This is well seen in *Experiment I*,[2] in which the influence of saponin on the conductivity of unhardened blood is compared with that on blood fixed by formaldehyde. λ, as in the other experiments, is an abbreviation for $\lambda\ (5^\circ) \times 10^5$. The conductivities are expressed in reciprocal ohms reduced to 5° C. The measurements were made by Kohlrausch's telephone method, the tube containing the blood being immersed in running water, the temperature of which did not vary more than 2° or 3° from 5° C. The saponin solution used was made by dissolving 3 grammes crude quillaia saponin in salt solution and adding salt solution up to 100 cc. The salt solution had in some experiments a strength of about 0.7 per cent, in others of about 1 per cent. Its conductivity was always carefully measured, as was also that of the saponin solution, which did not differ much from the conductivity of the salt solution. As a control, observations were also made on blood to which, instead of saponin solution, a similar amount of the salt solution was added.

It will be seen that in *Exp. I*, in the observation of April 18, the serum from the mixture of A (the formaldehyde blood) and saponin has λ 83.66, while the serum from the corresponding mixture of A with the sodium chloride solution has λ 78.48. A portion even of the small difference shown here is accounted for by the greater conductivity of the saponin than of the sodium chloride solution. On the other hand, for the sediment of corpuscles from the mixture of A and saponin, after prolonged centrifugalization, λ is 46.51 against 18.68 for the sediment of the control experiment with A and sodium chloride, and 14.76 for the sediment of A. The great difference between the sediments is certainly not to be explained by the presence of a greater quantity of serum between the corpuscles in the sediment of the saponin mixture than in the others, for the sediment was exceedingly compact and well-separated from the serum. There can be no doubt, therefore, that saponin does render the corpuscles better conductors. From this it necessarily follows

[2] The protocols of experiments are at the end of this article (p. 272).

that the "envelope" of the corpuscle is rendered permeable to ions to which it refuses passage before the action of the saponin, or more rapidly permeable to those which penetrate it only slowly and with difficulty. But the experiment does not enable us to say whether, in addition, the saponin produces an increased dissociation inside the corpuscle.

I desire to point this out clearly because in a recent paper on the increase of conductivity which is known to occur in dying muscle,[3] it is tacitly assumed that an increase of conductivity necessarily implies the formation of an increased number of ions in the muscle and is proportional to the increase in the number. Now while it is, of course, a perfectly familiar fact that when muscle dies a decomposition takes places which leads to the formation of substances capable of acting as conductors, we must not assume that this is the only way in which the conductivity is increased, until it is shown that no change in the resistance of the membranes through which the ions must pass is produced at death. The fact that the resistance of muscle is much greater in the transverse than in the longitudinal direction suggests very strongly that the resistance of the sarcolemma, and possibly also of whatever "envelopes" enclose the sarcostyles, is greater than that of the conducting liquids between the fibres or between the fibrils. And if those "membranes" are relatively impermeable to the electrolytes during the life of the tissue, as they should be if the proper osmotic relations are to be preserved, it is quite probable that their permeability, and therefore their resistance, becomes altered when the tissue dies. In this connection the fact mentioned in my previous paper, that perfectly fresh colored corpuscles are far less permeable to NH_4Cl than corpuscles which have stood for a few hours seems very suggestive.

I may mention here another observation which supports the conclusion that the envelopes of the corpuscles are altered by saponin. It was invariably found that when saponin was added at such an interval after the addition of formaldehyde that it no longer caused laking, the sediment of corpuscles settled far more slowly and less

[3] T. Kodis, *American Journ. of Physiology*, 1901, v, p. 267.

perfectly, was always more bulky in proportion to the liquid above it, and formed a less compact and tenacious mass on the bottom of the test-tube than the sediment from formaldehyde blood which had not been treated with saponin or to which NaCl solution had been added in the same proportion as the saponin solution. This refers to the sediment obtained when the blood was simply allowed to stand in a cold room and not to that separated by prolonged centrifugalization. It was always much easier to shake up into complete suspension in the serum the sediment of the formaldehyde blood which had been acted on by saponin; and the greater the amount of saponin solution added, the looser and more easily shaken up was the sediment. The same effect was observed, though it was not so pronounced, in blood to which NH_4Cl solution was added in such amount as to cause partial laking, and which was then treated with formaldehyde. The sediment was distinctly looser than when NaCl was substituted for NH_4Cl in this combination. The suggestion is that NH_4Cl alters the envelopes of the corpuscles in such a way that they are less liable to adhere firmly to each other when acted on by formaldehyde, and that saponin undoes or lessens the formaldehyde action which causes them to adhere. The fact that NH_4Cl penetrates the corpuscles much more readily than NaCl also shows that its relation to the envelope is different. In this experiment it is incidentally shown that the preference of the corpuscles for NH_4Cl as compared with NaCl is still evident 15 days after the addition of formaldehyde to the blood.

Some leucocytes are of course always present in the sediments whose conductivity is compared in the experiment, which, however, yields no information as to the action of saponin upon these. But some observations on pus, to be presently described, show that saponin affects the conductivity of pus corpuscles in very much the same way as that of the red corpuscles. Since, as I have previously shown, the action of saponin on the red corpuscles is essentially the same whether they are perfectly fresh or have stood for days, it seems highly probable that the conductivity of the leucocytes of blood is also increased by saponin, and in the same way, that is, by an increase of their permeability to the ions of the serum. The "envelope" of

the leucocyte (or at least of the pus corpuscle), however, differs from that of the colored corpuscle, as we shall see, in exhibiting no preference for NH_4Cl as compared with NaCl.

In *Experiment II* the effect of varying the quantity of saponin added to formaldehyde blood on its conductivity was investigated. Under λ the conductivities of the mixtures of formaldehyde blood and saponin are given, and in the column headed "λ of control" the conductivities of mixtures of the formaldehyde blood with similar quantities of the NaCl solution used in making the saponin solution. In the third column the differences between λ and λ of control are given, that is to say, the approximate amounts by which the conductivity of the blood has been increased by the action of the saponin, after deducting the increase due to the NaCl in the saponin solution. I shall speak of the numbers in this column as the true saponin effect.

The experiment shows that the addition of 1cc. of the saponin solution (the largest quantity used) to 5 cc. of the formaldehyde blood (A) causes practically the same true saponin effect as the addition of 0.4 cc. of the saponin solution to 5 cc. of A. In both cases, too, the full increase of conductivity is already developed at the time of the first measurement (a few minutes after mixture), and there is no further increase when the mixture is allowed to stand for many hours. When 0.2 cc. of the saponin solution is added to 5 cc. of A the true saponin effect ultimately becomes just as great as when 0.4 cc. or 1 cc. is added, but it has not reached its maximum 12 minutes after mixture. Eight hours after mixture the effect is complete, and there is no further increase after many hours. With 0.1 cc. of saponin solution to 5 cc. of A the true saponin effect is only 14.79, 20 minutes after mixture, as compared with 22.14, 19 minutes after the addition of 0.4 cc. of saponin solution to 5 cc. of A. But in the observation with 0.1 cc. of saponin solution the effect has not reached its maximum even 2 hours and 20 minutes after mixture, when it is 16.23. In 19 hours more it has further increased to 18.05. With 0.05 cc. of the saponin solution to 5 cc. of A the increase in the true saponin effect, as time goes on, is still more striking, the effect being only 5.39, 27 minutes after mixture, but 8.56, $4\frac{1}{2}$ hours after mixture, and 9.31,

16 hours later. The maximum effect remains much less for this quantity of saponin than for 0.1 cc. or anything above it. With 0.02 cc. of the saponin solution (the smallest quantity employed) to 5 cc. of A the maximum effect is still less, but the relative increase between the first and last measurement is the greatest of all (from 0.74 to 3.33).

The action of saponin in laking the colored corpuscles has usually been ascribed to a solution or "corrosion" of some constituent of the stroma (or envelope). The fact that it greatly increases the conductivity of the formaldehyde-hardened corpuscles is an additional argument in favor of this view. It further shows that the substance on which saponin acts is not fixed by formaldehyde, within the longest period investigated (a fortnight), or at least is not altered in such a way as to become incapable of being attacked by saponin. This makes it highly probable that the substance is not a proteid. Ransom[1] has recently brought forward evidence that the substance on which saponin acts when it lakes the corpuscles is cholesterin. My results are compatible with this idea. But it seems certain that if the saponin produces its effect on the conductivity of formaldehyde blood by attacking the cholesterin in the corpuscles, the result is due not to any merely chemical reaction between the saponin and the cholesterin, but to a change of structure in the corpuscles which renders them more permeable to ions and perhaps in addition to an increased dissociation of electrolytes in the corpuscles. I have not hitherto found anything in the appearance of the corpuscles under the microscope or their behavior to stains which throws any light on the point of attack of the saponin.

In *Experiment III* I endeavored to determine whether the addition of saponin to yolk of egg, which is known to be rich in cholesterin (and lecithin) compounds would affect the conductivity at all in the same way as it affects the conductivity of blood. I found that it had no more influence on the yolk than on the white, and no greater effect on either than was due to the electrolytes contained in the saponin solution. On the other hand it was shown that there was a marked difference in the effect of dilution with water on the conduc-

[1] *Deutsche med. Wochensch.*, 1901, p. 194.

tivity of the white and the yolk respectively, the dilution causing a much smaller proportional diminution in the conductivity of the yolk than in that of the white. Thus in *Experiment IV* the conductivity of the yolk was 19.83 and that of the white 56.46. Dilution with 1 volume of water (Fig. 1) reduced the conductivity of the former to 17.76 and that of the latter to 33.98; while the addition of 3 volumes of water made the conductivity of the yolk 11.52 and that of the white 19.26. The conductivity of the undiluted yolk is always much less than that of the undiluted white, the ratio being usually about 1:3.

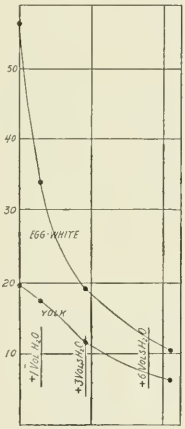


FIG. 1.

Curves showing the effect of dilution with water on the conductivity of yolk and of white of egg, plotted from Exp. IV. Conductivities laid off along vertical axis, and quantities of water added along horizontal axis.

The fact that saponin increases the conductivity of blood-corpuscles which have been fixed by formaldehyde suggests that its primary action on unhardened blood is the same, viz., an increase in the permeability of the envelope to the electrolytes of the serum or of the corpuscles themselves. The osmotic equilibrium would be thus

upset: some of the water of the serum would pass into the corpuscles; and when the increase of permeability reached a certain limit enough water might enter to cause of itself the discharge of the hæmoglobin. Saponin-laking would thus be reduced to a special form of water-laking, as Nolf[5] and Hédon[6] have suggested. My experiments, in so far as they demonstrate an actual increase of permeability for the electrolytes, are distinctly in favor of this hypothesis. Support is also lent to this view by the fact that an amount of saponin insufficient to produce laking may cause the blood to darken, the corpuscles swelling up without losing hæmoglobin. This change is not accompanied by the marked increase of conductivity which signalizes the actual onset of the laking process, although the conductivity may be somewhat increased.

Another fact, which, if it does not so directly support this theory of the saponin action, is at least consistent with it, is that the laking action of saponin and also its effect on the conductivity are much more easily produced at a temperature of 40° to 45° C. than at the ordinary temperature. A dose of saponin which may be ineffective at room temperature may cause complete laking and the characteristic increase of conductivity when the blood is heated to the temperature mentioned, and a dose which may be just capable of laking slowly at ordinary temperature will bring about rapid laking at 40° to 45°. Occasionally I have noticed, as in *Exp. V*, that a dose to which blood is refractory at 40° to 45°, becomes rapidly effective at 50° C. The phenomenon is different from heat-laking, for this cannot be obtained even by prolonged heating at such temperatures. The action seems to be simply the ordinary saponin action intensified by the increase of temperature, which may diminish the power of resistance of the envelope to the attack of the saponin and the penetration of the electrolytes.

A diminution in the resistance of the corpuscles to the action of saponin may be actually observed when blood is allowed to stand for a while before addition of the saponin. A given dose of saponin may

[5] *Ann. de l'Institut Pasteur*, 1900, p. 297.

Archives internat. de pharmacodynamie et de thérapie, 1901, viii, p. 403.

then produce a greater increase of conductivity and more complete laking than the same dose produced in the fresh blood; and a dose which is insufficient to bring about complete laking, with the corresponding increase of conductivity, in fresh blood, may do so in stale blood.

With the view of throwing further light on the changes which occur in the serum and the corpuscles when unhardened blood is laked by saponin—a subject, as already remarked, less easy to study than the corresponding changes in formaldehyde blood—I have compared the conductivity and freezing-point of ordinary defibrinated blood and of formaldehyde blood before and after the addition of saponin. The same quantities were determined for the sera of the defibrinated blood, the formaldehyde blood and the mixture of the latter with saponin (*Experiment V*). It was found impracticable to separate the ghosts from the saponin-laked defibrinated blood by the centrifuge, and therefore in this case the freezing-point was measured directly on the laked blood, and the conductivity of the corpuscle-free liquid was not determined. But in *Experiment VI* the ghosts were completely separated from saponin-laked blood by filtration through a pot of porous clay, and the conductivity of the liquid measured.

In *Exp. V* from the percentage of serum in the defibrinated blood (estimated by the electrical method previously described by me[7]) and the actual Δ of the serum and saponin solution I have calculated the value of Δ for the extra-corpuscular liquid of the mixture of blood and saponin, on the assumption that no exchange of water or dissolved solids between the corpuscles and this liquid is caused by the saponin, or that, if there is any exchange, water accompanies the solids in such proportion as to leave the Δ of the extra-corpuscular liquid the same as if no exchange took place.

From the percentage of extra-corpuscular liquid[8] in the formaldehyde

[7] *Journ. of Physiology*, 1899, xxiv, p. 356.

[8] I have supposed for the purpose of calculating this percentage that the formaldehyde solution when added to blood simply mixes with the serum, without any alteration in the volume of the serum-formaldehyde mixture taking place through exchange with the corpuscles. Since the corpuscles preserve their normal size and shape this assumption is approximately correct. But although there is no notable movement of

blood A and the actual λ and Δ of this liquid and of the saponin solution I have, on the same assumption, calculated the value of λ and Δ for the extra-corpuscular liquid of the mixture of A and saponin. In calculating λ and Δ I have made no allowance for possible changes in the dissociation coefficient when mixture takes place. Comparison of the actual and calculated values of λ and Δ, of course, affords information as to the correctness of the assumption on which the calculation is based, and where it is erroneous indicates how it must be modified.

It will be seen that for the mixture of defibrinated blood and saponin the actual is distinctly greater than the calculated Δ. This is doubtless due in part to the escape of hæmoglobin into the serum. But the molecular weight of hæmoglobin is so great that even the solution of the whole of the hæmoglobin would not suffice to account for the difference. In *Experiment VI* the liquid obtained by filtering off the ghosts from saponin-laked blood had a conductivity about equal to that of the serum of the original defibrinated blood, although decidedly less than that of serum obtained from blood to which as much of the NaCl solution used in making the saponin solution had been added as was added of the saponin solution to the saponin blood. The deficiency in λ of the serum of the saponin-laked blood is easily explained by the depressing influence on the conductivity exerted by the dissolved hæmoglobin. Indeed the quantity of hæmoglobin in solution would cause a considerably greater deficiency than actually exists. So that it seems necessary to suppose that water has entered the corpuscles from the serum in greater proportion than electrolytes, or that if water has come out of the corpuscles it has been accompanied by electrolytes in such amount that, apart from the depressing influence of the hæmoglobin, the conductivity of the serum would have been increased. The latter alternative would also explain the excess of the actual over the calculated Δ in *Exp. V*, and it is this that we must accept. For although at the beginning of the saponin action water seems to enter the corpuscles and to cause them to swell, the process must be reversed with the discharge of the hæmoglobin, since in saponin-laked blood the ghosts constitute only a relatively small proportion of the total volume. We must, therefore, suppose that as the hæmoglobin escapes from the corpuscles, they also lose water and electrolytes.

water into or out of the corpuscles, much of the formaldehyde certainly enters them. This is well illustrated by the fact that for the serum from A the calculated is much greater than the actual Δ, while the actual γ is almost the same as the calculated. This shows that the difference between the actual and calculated Δ is due to the passage of non-electrolytes, and not of electrolytes into the corpuscles.

In the case of the extra-corpuscular liquid of the mixture of formaldehyde blood and saponin the difference between the actual and calculated Δ is too great to be explained by the passage even of all the saponin into the corpuscles. It is most easily accounted for by the supposition that saponin increases the permeability of the corpuscles for certain of the dissolved substances in the extra-corpuscular liquid. If any salts pass into the corpuscles, water must enter along with them, or other salts must escape from the corpuscles, since the actual λ of the corpuscle-free liquid (referred to as "top" in the tables) is practically the same as the calculated λ. The difference between the actual and calculated Δ must accordingly be due to the passage not of electrolytes but of non-electrolytes (formaldehyde) into the corpuscles. As has been shown in *Exp. I*, the marked increase of λ of the entire formaldehyde blood under the influence of saponin is due to an increase in the permeability of the corpuscles for ions. I have already given reasons for supposing that when ordinary (unhardened) blood is acted on by saponin the corpuscles become more permeable to the salts of the serum, or certain of them, and presumably to their ions. Whether this increase of permeability persists when the corpuscles have been reduced to ghosts by the escape of the hæmoglobin I am unable to say. The ghosts certainly remain worse conductors than the serum, as is shown by the fact that as the serum is separated from saponin-laked blood the conductivity of the residue sinks (see e. g. *Exp. VI*).

Another point of interest illustrated in *Exp. V*, and still better in *Exp. I*, is that the conductivity of a specimen of defibrinated blood treated first with formaldehyde and then after a while with saponin is not in general the same as the conductivity of another specimen of the same blood to which saponin is first added and then formaldehyde in the same amount as in the first observation. The difference, of course, depends on the presence of the corpuscles, and would not be seen if the electrolytes of the blood were all in simple solution. The combination blood + saponin + formaldehyde has in general a smaller conductivity than the combination blood + formaldehyde + saponin, especially when in the second combination blood which has been acted on so long by the formaldehyde that no laking takes place on the addition of saponin is employed. This supports the conclusion already drawn, that one of the causes of the smaller conductivity of the first combination is the depressing effect of the hæmoglobin on the conductivity of the laked blood. In addition, it is probable that a great part of the formaldehyde, which, as a non-conductor, depresses the conduc-

tivity of the extra-corpuscular liquid, is in the second combination fixed in the corpuscles.

THE BEHAVIOR OF PUS CELLS TO NH_4Cl, NaCl, SAPONIN AND WATER.

In order to see whether other cells, and especially dead cells, would exhibit any of the peculiarities studied in the colored corpuscles, I made some experiments with pus.

In *Experiment VII* it is shown that pus corpuscles, like colored blood-corpuscles, have a smaller conductivity than the serum of pus. The serum can be separated from the corpuscles by filtering the pus through porous clay, but more easily, and as completely, or nearly so, by filtering it through paper, putting the first portion of the filtrate back on the filter. I have had no success in obtaining the serum free from corpuscles by the centrifuge. On the average the conductivity of the pus serum is very much the same as that of blood serum, but sometimes it is considerably greater, especially, of course, if putrefaction is at all pronounced. I have preciously shown[9] that in putrefying liquids the conductivity increases and the freezing-point diminishes progressively up to a certain limit, as time goes on, owing to the conversion of proteids or other complex substances into simpler compounds, some of which are electrolytes. I have never obtained such small values for the conductivity of sediments rich in pus cells as for blood sediments, and, therefore, it would seem that pus corpuscles are more permeable to the ions of the serum than blood-corpuscles are. But, as is seen in *Exp. VII*, the conductivity of the sediment is much less than that of the serum.

When diluted with water (*Exp. VII* and Fig. 2) pus has a higher conductivity than a simple solution of electrolytes or blood-serum or egg-white diluted to the same extent, but a lower conductivity than correspondingly diluted yolk of egg (*Exp. IV* and Fig. 1) or defibrinated blood. This indicates that electrolytes may pass out of the corpuscles when pus is diluted with water. But in the absence of measurements of the conductivity and freezing point of the corpuscle-free liquid from the diluted pus one cannot make any positive statement as to this. For there are three other factors which would cause

[9] *Journal of Experimental Medicine*, 1899, iv, p. 235.

a relative increase of conductivity: increased dissociation of electrolytes in the serum, diminution of the depressing effect of the non-electrolytes, and increase in the average breadth of the conducting paths between the corpuscles.

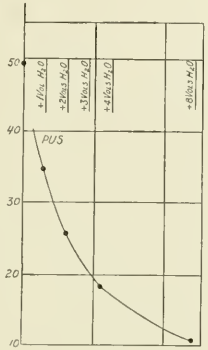


FIG. 2.

Curve showing the effect of dilution with water on the conductivity of pus, plotted from Exp. VII. Conductivities along vertical, quantities of water added along horizontal axis.

Heating pus to 69° C. (*Exp. VII*) and subsequent dilution failed to reveal any analogy between the behavior of pus corpuscles and that of blood corpuscles, the ratio between the conductivity of the pus which had been heated and that of the heated pus after dilution with its own volume of water being practically the same as the corresponding ratio for a specimen of unheated pus and for a sediment of pus. In *Exp. IX* it is shown that when pus is heated to 80° C. for 10 minutes its conductivity is increased. The increase is still greater when the pus is heated to 93° for 15 minutes, although the conductivity of the serum is diminished.

On the other hand, saponin, as is shown in *Experiment VIII*, increases the conductivity of pus just as it does the conductivity of blood. This is the case also when saponin is added to pus which has been treated with formaldehyde. The action is on the corpuscles, since the

addition of saponin to pus serum causes only the change of conductivity due to the electrolytes in the saponin solution (*Exp. IX*). When saponin is added to pus which has been heated to 93° C. (*Exp. IX*) the characteristic increase of conductivity is still produced, although the proteids of the corpuscles must have been coagulated. With the microscope the corpuscles, or at least great numbers of them, were seen to be still intact, and stained normally. These facts are in accordance with what has been already mentioned as to the action of saponin on formaldehyde blood, and suggest that in the case of pus also the constituent of the corpuscles on which the saponin acts is not of proteid nature.

At the same time it ought to be pointed out that the entire increase produced by saponin in the conductivity of the heated pus is not due to an action on the corpuscles; a portion of the increase is caused by some action of the saponin on the serum. This is proved in *Experiment IX*, where the behavior to saponin of the serum of unheated pus is compared with that of the serum of the same pus after heating to 93°. In the case of the serum of the unheated pus the saponin, as already stated, produces only the small increase of conductivity corresponding to the electrolytes in the saponin solution. The increase in the conductivity of the serum of the heated pus is too great to be entirely accounted for in this way. I have no results as yet which would enable us to decide whether the action of the saponin on the serum of the heated pus causes the granules present in it (some of which may have escaped from corpuscles either partially or wholly broken down by the heating) to become better conductors, or whether the increase in conductivity is due to the action of the saponin on dissolved substances. It cannot be due to any action on intact corpuscles in the serum, for it was shown by the microscope that none such were present.

There is no evidence (*Exp. VIII*) that pus corpuscles take up NH_4Cl in preference to NaCl either in the case of normal pus or of pus hardened by formaldehyde.

SUMMARY.

1. The increase of conductivity produced by saponin in formaldehyde-hardened blood is due to an increase in the conductivity of the

corpuscles (increased permeability of the corpuscles to ions) and not, mainly at any rate, to the liberation of electrolytes from the corpuscles and a consequent increase in the conductivity of the serum. The increase in the permeability of the corpuscles is probably caused by a "corrosive," dissolving, or emulsifying action of the saponin on some non-proteid constituent of the envelope or stroma.

2. In the first stage of the action of saponin on blood (not fixed by formaldehyde) there seems also to be an increase in the permeability of the corpuscles for ions, even before any hæmoglobin has been liberated. The liberation of the hæmoglobin may be secondary to this, owing to the entrance of water consequent on the disturbance of osmotic equilibrium.

3. Heating the blood to 40° to 45° C. intensifies the laking action of saponin, so that a dose insufficient to cause laking at ordinary temperature may do so when the blood is heated to the temperature mentioned.

4. Pus corpuscles, like red blood corpuscles, are worse conductors than the serum in which they are suspended. Unlike blood corpuscles, they show no preference for NH_4Cl as compared with NaCl. On the other hand, the conductivity of pus is increased by the action of saponin, just as is the case with blood, and apparently very much in the same way, that is to say, by an action on the corpuscles and not on the serum. The fixing of the pus corpuscles by formaldehyde does not hinder this action of saponin.

EXP. I.

April 17. At 10.20 A. M. obtained defibrinated blood from a bitch. At 3.50 P. M. added to 150 cc. of the defibrinated blood 75 cc. of a 4 per cent solution of formaldehyde in NaCl solution. The mixture is called A. For the formaldehyde solution $\lambda = 64.18$; for the saponin solution $\lambda = 83.94$; for the NaCl used in making the saponin and formaldehyde solutions $\lambda = 78.97$. Percentage of serum in the defibrinated blood calculated by the electrical method, 51.5.

Time.	Defibrinated blood.	λ
April 17, P. M.		
8.30	Defibrinated blood	30.18
7.17	20 cc. defib. blood + 1.6 cc. saponin.	
7.23		34.63
7.45	Not laked, only somewhat darkened; heated in bath at 44° to 45° along with A + saponin. A + sapo. lakes far sooner and more completely than defib. blood + saponin.	
8.10	Defib. blood + saponin (well laked).	37.95
8.14	To the mixture of defib. blood and saponin added 10 cc. formald. sol.	
8.45	This mixture	43.71
	(After standing 18 hours)	44.55
April 18, P. M.		
3.05	Defibrinated blood	28.87
	Serum from clot	84.22
2.55	20 cc. blood + 1.6 cc. saponin.	
3.17	Only somewhat darkened; not at all laked.	
4.25	Not laked, although dark.	
6.15	Now laked	43.11
6.20	Added to this mixture of defib. blood and saponin 10 cc. formald. sol.	
6.37	This mixture	47.56
	(After standing 13 hrs., perfectly laked and still thin)	46.86
	Bottom* (after centrif. 20 min.).	47.47
3.17	20 cc. blood + 1.6 cc. of the NaCl sol. used in making the saponin sol.	
6.05		32.09
	(After standing 13½ hrs.)	29.55

Time.	Formaldehyde blood A.	λ
April 17, 8.37 P.M.	A	37.01
7.38	30 cc. A + 1.6 cc. saponin sol.	
7.45	Considerably darkened.	
8.23	Well laked	56.33
	(After standing 18 hrs. is thick and viscid)	53.26
April 18, P. M.		
3.33	30 cc. A + 1.6 cc. saponin sol.	
6.20	Not laked	58.41
	(After standing 13 hrs. is still thin and not laked)	59.79
	Top (after centrif. for 50 min.)	83.66
	Bottom	46.51
	A + as much of the NaCl sol. used in making the sapo. solution as was added of the saponin sol.	
	(After standing 22 hrs.)	41.90
	Top (after centrif. for 20 min)	78.48
	Bottom	18.68
	Top of A (after centrif. for 50 m.)	79.72
	Bottom	14.76
April 19, 8.01 P.M.	15 cc. A + 1.2 cc. saponin sol.	
8.05		58.95
	(After standing 18 hrs.)	61.38
	Top (after centrif.)	80.23
	Bottom	51.42
8.12	10 cc. A + 20 cc. water.	
8.17		23.02
	(After standing 17 hrs.)	23.78
	Top	30.11
	Bottom	13.60
	Bottom (after centrif.)	7.52
8.25	A	35.96
May 2. A. M.		
10.49	A	38.53
11.00	5 cc. A + 0.4 cc. saponin sol.†	
11.19		66.53
P. M.		
7.53		66.19
	(19 hours later)	66.36
A. M.		
11.00	5 cc. A + 0.4 cc. of the NaCl sol.‡ used in making the saponin sol.	
11.11		44.09
11.20	5 cc. A + 5 cc. NH_4Cl§	
	(After standing 24 hours	74.34
	Top	103.47
11.20	5 cc. A + 5 cc. NaCl ‡	
	(After standing 24 hours)	80.23
	Top	111.18

* There is very little separation. The ghosts cannot be separated by the centrifuge but can be completely separated by filtration through paper. After a time both the filtrate and the sediment on the filter set into a very firm jelly.

† For this saponin solution $\lambda = 139.30$.
‡ For the NaCl solution $\lambda = 140.07$.
§ For the NH_4Cl solution $\lambda = 143.10$.

EXP. II.

To show effect of different quantities of saponin on the conductivity of formaldehyde blood. Formaldehyde blood A (from Exp. V) was used. On April 17, 75 cc. of a 4 per cent solution of formaldehyde in NaCl solution was added to 150 cc. of bitch's defibrinated blood. For the saponin solution $\lambda = 139.30$. For the NaCl solution used in making the saponin solution $\lambda = 140.07$.

Time.		λ	λ of control.	Difference bet. λ and λ of control.
May 2,				
10.49 A. M.	A	38.53		
11.00	5 cc. A + 0.4 cc. saponin.			
11.19		66.53	44.09	22.44
7.53 P. M.		66.19		22.10
	(19 hours later)	66.36		22.27
11.50 A. M.	5 cc. A + 0.2 cc. saponin.			
12.02		62.44	41.35	21.09
8.01 P. M.		63.85		22.50
	(19 hours later)	63.85		22.50
3.13 P. M.	5 cc. A + 0.1 cc. saponin.			
3.33		54.52	39.73	14.79
8.08		55.96		16.23
	(19 hours later)	57.78		18.05
3.50	5 cc. A + 0.05 cc. saponin.			
4.17		43.86	38.47	5.39
8.15		47.03		8.56
	(16 hours later)	48.19	38.88	9.31
3.50	5 cc. A + 1 cc. saponin.			
4.23		73.91	51.11	22.80
8.23		72.84		21.73
	(16 hours later)	72.84		21.73
4.35	5 cc. A + 0.02 cc. saponin.			
4.50		39.80	39.06	0.74
8.30		40.95		1.89
	(15½ hours later)	42.39		3.33

EXP. III.

White and yolk of two fresh hen's eggs, beaten up separately and strained through muslin. For the saponin solution $\lambda = 140.83$; for the NaCl solution used in making it, $\lambda = 141.62$.

	λ		λ
White	59.09	Yolk	21.45
5 cc. white + 0.8 cc. saponin solution	69.26	5 cc. yolk + 0.8 cc. saponin solution	35.06
5 cc. white + 0.81 cc.* of the NaCl sol. used in making the saponin sol.	69.07	5 cc. yolk + 0.81 cc.* of the NaCl sol. used in making the saponin sol.	35.36

* .01 cc. too much was added by mistake.

EXP. IV.

White and yolk of hen's egg beaten up separately.

	λ	Ratio of λ of undiluted to λ of diluted.		λ	Ratio of λ of undiluted to λ of diluted.
White	56.46		Yolk	19.83	
+ 1 vol. water	33.98	1.66	+ 1 vol. water	17.76	1.11
+ 3 " "	19.26	2.93	+ 3 " "	11.52	1.72
+ 7 " "	10.41	5.42	+ 7 " "	6.78	2.92

To dog's defibrinated blood, two hours after it was drawn, one-half of its volume of a 4 per cent solution of formaldehyde in a 1 per cent NaCl solution was added. Call the mixture A. The measurements in the table were begun 18 hours later. The animal had been used for intravenous injection of glycerine extract of suprarenal capsule one hour before the blood was obtained. For the formaldehyde solution $\lambda = 111.18$; $\Delta = 3.755$. For the NaCl solution $\lambda = 139.30$; $\Delta = 0.732$. For the saponin solution $\lambda = 134.84$; $\Delta = 0.851$. The proportion of serum in the defibrinated blood as calculated by the electrical method is 60.6 per cent. The proportion of serum (extracorpuscular liquid) in A, calculated on the assumption that the addition of the formaldehyde caused neither loss nor gain of water by the corpuscles, is 73.7 per cent.

DEFIBRINATED BLOOD.						FORMALDEHYDE BLOOD (A).					
Time.		λ actual.	λ calculated.	Δ actual.	Δ calculated.	Time.		λ actual.	λ calculated.	Δ actual.	Δ calculated.
P. M.						P. M.					
1.18	Serum from defibrinated blood..	85.93		0.761		1.32	Serum from A (practically free from blood pigment).....	96.75	97.3	1.686	2.11
4.22	Defibrinated blood.....	38.47				2.21	A	61.53			
2.59	To 20 cc. of defib. blood added 1.6 cc. saponin solution.					2.27	To 25 cc. of A added 1.33 cc. of the saponin sol.				
6.10	No laking. Added 0.4 cc. more of the saponin solution.					6.10	No laking. Added 0.4 cc. of the saponin sol.				
6.35	Not laked. Heated to 44° for 15 min. Darkens somewhat, but is not laked.					6.35	Not laked. Heated to 44° for 15 min. No change.				
6.50	Added 0.4 cc. more of the sapo. sol. Left at 45° for 16 min.; is darkened but not laked.					6.50	Added 0.4 cc. more of the saponin solution, and left in bath at 45° for 16 min.				
7.25	Added 0.4 cc. more of the saponin solution.					7.25	Added 0.4 cc. more of the saponin solution.				
7.35	Not laked. Heated to 50° for 10 min.; lakes almost at once.					7.35	Not laked. Heated to 50° for 10 min.; although darker than A, it is not laked.				
7.54	Seems perfectly laked.....	56.58									
	(After standing 23 hrs. more)...	66.36		0.799	.778	8.05	Not laked	80.22			
	Serum from mixture of blood and saponin		95.10				(After standing 15½ hrs. more).	81.25			
8.35	To 11.4 cc. of the mixture of blood and saponin added 5 cc. of the formaldehyde solution.						Top (after centrifugalization).	102.22	101.3	1.427	1.585
9.03	(Quite thick, but can still be poured out).....	75.00			1.97						
	In 4 min. more it set completely into a jelly, so that Δ could not be determined.										
	Top.....		101.5		1.97						

EXP. VI.

Dog's defibrinated blood. For the saponin solution $\lambda = 140.83$; for the NaCl solution used in making it $\lambda = 141.62$.

	λ
The defibrinated blood	33.75
Serum from clot (free from haemoglobin)	88.33
To 50 cc. of defibrinated blood added 4 cc. of saponin solution (dark and laked in 5 minutes)	68.51
Filtered the saponin blood several times through paper, and centrifugalized the filtrate.	
Filtrate (contains many ghosts and leucocytes)	71.01
Filtered this filtrate through a pot of porous clay.* (Now free from corpuscles)	88.64
Residue containing the ghosts and leucocytes but also much serum	67.78
To 25 cc. of defibrinated blood added 2 cc. of the NaCl solution used in making the saponin solution. This mixture	38.47
Serum from this mixture (free from corpuscles)	98.26

* It filters much more slowly than water-laked blood, obtained by adding 2 volumes water to the same defibrinated blood, and filtered under the same pressure.

EXP. VII.

	λ	Ratio of λ of pus to λ of diluted pus.
Pus from tubercular hip joint	49.51	
+1 vol. water	34.12	1.45
+2 "	25.66	1.92
+3½ "	18.56	2.66
+8 "	10.39	4.75
Clear serum from pus (filtered through clay)	86.52	
Fresh pus obtained on another occasion from the same hip joint	57.61	
Clear serum (filtered through paper)	77.52	
Sediment	56.58	
Sediment + 1 vol. water	39.45	1.43
" +3 vols. water	22.04	2.56
Another specimen of pus	49.56	
Clear serum from it (filtered through paper)	91.30	
Sediment	38.55	
Another specimen of pus	49.94	
Heated to 69° C. (became thicker than original pus)	50.49	
Heated pus + 1 vol. water	34.87	1.44

EXP. VIII.

Pus from a case of empyema obtained May 7. On May 8, at 8 P. M., added to 50 cc. of the pus 20 cc. of a 4 per cent solution of formaldehyde in NaCl solution. Call the mixture A. For the formaldehyde solution $\lambda=123.66$. For the saponin sol. $\lambda=140.83$; for the NaCl solution $\lambda=141.62$; for the NH_4Cl solution $\lambda=143.90$.

Time.	Pus.	λ	Time.	Formaldehyde pus (A).	λ
May 8, P. M.			May 9, P. M.		
8.09	Pus	46.51	4.26	A	66.19
	Serum separated by filtration	109.27		Sernm from A	93.54
7.36	10 cc. pus + 10 cc. NH_4Cl.		4.40	10 cc. of A + 10 cc. NH_4Cl.	
8.19		89.58	7.19		101.81
	(After standing 12 hours more)	89.26	4.40	10 cc. of A + 10 cc. NaCl.	
	Top	117.91	7.25		101.00
7.37	10 cc. pus + 10 cc. NaCl.		4.49	5 cc. of A + 0.4 cc. saponin.	
8.25		89.26	7.32		78.48
	(After standing 20 hours more)	88.64	4.49	5 cc. of A + 0.4 cc. of the NaCl solution used in making the saponin sol.	
	Top	116.28			
7.41	10 cc. pus + 0.8 cc. saponin.				
8.31		59.65	7.40		70.03
	(After standing 11½ hrs. more)	61.98	4.52	5 cc. of A + 10 cc. of water.	
7.42	10 cc. pus + 0.8 cc. of the NaCl sol. used in makiug the saponin solution.		7.48		32.67
				(After standing 16 hrs.) Top.	38.82
			May 14, A. M.		
8.37		52.27			
7.45	10 cc. pus + 20 cc. water.		11.47	5 cc. of A + 0.4 cc. of saponin.	
8.49		29.34	P. M.		
9.10	10 cc. pus + 1.6 cc. saponin.		12.07		80.47
	(After standing 10½ hrs.)	74.34	A. M.		
9.10	10 cc. pus + 1.6 cc. of the NaCl sol. used in making the saponin solution.		11.47	5 cc. of A + 0.4 cc. of the NaCl solution used in making the saponin sol.	
	(After standing 10½ hrs.)	56.08	12.00		71.41

EXP. IX.

Same pus as that used in Exp. VIII obtained on May 7. The solutions of NH_4Cl, and NaCl, saponin are the same as those used in Exp. VIII.

Time.		λ	λ of control *
May 14,			
3.00 P. M.	Pus heated to 80° C. for 10 minutes	56.71	
3.10	5 cc. of the heated pus + 0.4 cc. saponin.		
3.28		63.69	61.23
3.48	The original pus (not heated)	52.81	
4.00	Pus heated to 93° C. for 10 minutes	61.23	
4.20	5 cc. of this heated pus + 0.4 cc. saponin.		
4.41		68.14	64.67
May 15,			
2.30 P. M.	Clear serum from original pus (filtered through paper, entirely free from pus corpuscles)	109.27	
2.42	5 cc. of this serum + 0.4 cc. saponin.		
2.59		111.67	111.67
7.21	Serum from pus which was heated to 93° (separated by centrifuge. Very few pus corpuscles in it, but a good many granules)	95.66	
7.16	5 cc. of this serum + 0.4 cc. saponin.		
7.45		105.63	101.81
May 16,			
10.53 A. M.	Serum from pus which was heated to 93° (separated by filtration through paper. Entirely free from pus corpuscles. Not many granules can be seen till methylene blue is added, when a good many appear, many of which are cocci)	93.54	
11.10	5 cc. of this serum + 0.4 cc. saponin.		
11.26		106.51	98.26

* In this column are given the conductivities of pus or serum of pus to which instead of saponin solution the corresponding amount of the NaCl solution used in making the saponin solution was added as a control.

SNAKE VENOM IN RELATION TO HÆMOLYSIS, BACTERIOLYSIS, AND TOXICITY.

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INTRODUCTION. GENERAL CONSIDERATIONS CONCERNING HÆMOLYSIS AND BACTERIOLYSIS.

[I have long desired that the action of venoms upon blood should be further examined. I finally indicated in a series of propositions the direction I wished the inquiry to take. Starting from these the following very satisfactory study has been made by Professor Flexner and Dr. Noguchi. My own share in it, although so limited, I mention with satisfaction.—S. Weir Mitchell.]

The following research, which is presented at this time in abstract, was conducted under a grant from the Bache fund of the National Academy of Sciences. It forms the first instalment of a new study

of venoms upon which we have been engaged during a year past and which, it is hoped, will be continued during another year or longer.

While these studies are still incomplete, the data here given have been worked out in detail and may therefore be accepted as final. On account of the large number of tables which will be given in the final publication, and the many drawings necessary to illustrate properly the text, more or less delay in bringing out the full work will be inevitable. But inasmuch as the results of the studies form an integral part of the work on hæmolysis and bacteriolysis, which is now attracting so much attention among bacteriologists and pathologists, and as they contain certain facts of fundamental importance bearing on the theory of these phenomena, it seems best not to delay publication until the entire series of researches shall have been completed.

At present we shall not give the full bibliography. Since the fundamental studies of Weir Mitchell and his collaborators,[1] the effects of venom upon the blood and the nervous system of animals have been generally recognized. The rapid putrefaction which sets in after poisoning with venom was also explained by Welch and Ewing's[2] observations on the loss of bactericidal power of the serum of such poisoned animals. The close relationship of the poison with certain toxins of bacteria and of higher plants was shown by the discovery of Sewall,[3] of Calmette[4] and of Fraser[5] that animals could be immunized from the effects of venom and that they yielded an active antitoxin. That the poison of venom is not simple, but that it consists of a complex of constituents of a proteid nature was proven by Mitchell and Reichert. The time, therefore, seemed ripe for a further study of the physiological effects of venom upon the blood, upon bacterial life, and upon tissues, in the light of the recent studies upon various kinds of immunity.

For the purpose of these studies dried venom has been employed.

[1] *Smithsonian Contrib. to Knowledge*, 1860, xii, and 1886, No. 647.
[2] *Lancet*, 1894, i, p. 1236.
[3] *Journal of Physiology*, 1887, viii, p. 203.
[4] *Ann. de l'Institut Pasteur*, 1894, viii, p. 275.
[5] *British Med. Journ.*, 1895, i, p. 1309.

Fortunately several kinds were available—through the kindness of Prof. Reichert, that of the rattlesnake (*Crotalus adamanteus*); of Dr. Joseph McFarland, that of the water mocassin (*Ancistrodon piscivorus*) and of the cobra (*Naja tripudians*), and of Messrs. Mulford & Co., that of the copperhead (*Ancistrodon contortrix*). We wish to express to these gentlemen our great appreciation of their kindness.

Before presenting the matter of our studies, it seems best to preface what we have to say with a brief statement of some of the facts and views relating to the phenomena of hæmolysis and bacteriolysis. In this preface we shall not distribute credits for the work or the views here embodied, inasmuch as this will be done in the complete publication. Only so much will be said as is necessary for an understanding of the experimental data relating to venom which are to follow.

By hæmolysis is meant solution of the blood-corpuscles. The term is usually applied to solution of the red corpuscles, but the white cells are also subject to a similar solution. It is, therefore, correct to speak of hæmolysis when both kinds of cells, erythrolysis when the red cells only, and leucolysis when the white cells alone are dissolved. In this solution of the red cells, which is the type of hæmolysis, the hæmoglobin is separated from the stroma of the corpuscles. The separation of hæmoglobin by hypotonic solutions and through the action of destructive chemical substances is not considered in this article. Hæmolysis as here employed refers to such separation through the action of complex agents derived from living plants or animals. This form is distinguished as biologic hæmolysis. Of all such agents the most active are found in the blood plasma or serum of alien animal species. Others are the products of cellular activity, such as venom, certain toxic products of bacterial growth, as tetanolysin, staphylotoxin, etc., and still others are yielded by some of the higher plants, as crotin from *Croton tiglium*.

The most familiar examples of hæmolysis are supplied by the effects of the transfusion of animal blood into man. It was early discovered that the practice was dangerous for the reason that the red corpuscles of the host were dissolved by the foreign blood. This effect was quickly seen to be due to the serum of the alien blood and it was observed to take place with equal readiness *in vitro*. The blood of animals

also is hæmolysed by foreign sera—the red corpuscles of the rabbit, for example, being dissolved readily by dog's serum. Some sera have very high dissolving power, the most active thus far known being that obtained from the eel, which is correspondingly toxic. After admixture of the corpuscles and foreign serum, solution does not occur immediately. The corpuscles first run together, become clumped, or, as we now denominate the effect, agglutinated. The dissolving effect of venom upon corpuscles is also preceded by a similar agglutination, as was first shown by Mitchell and Stewart.[6]

There is much similarity in the phenomena of agglutination and of lysis as observed in blood-corpuscles with appearances seen in connection with bacteria. The Gruber-Widal reaction of agglutination, which has served so well in the diagnosis of typhoid fever and other bacterial infectious diseases, is of a similar nature. Moreover, under certain conditions solution of the agglutinated bacteria may also occur, when bacteriolysis more or less analogous to hæmolysis results. The well-known Pfeiffer phenomenon, in which cholera spirilla undergo disintegration and solution in the peritoneal cavity of the immunized guinea-pig is the classical example of bacteriolysis.

The studies of the past two or three years upon the allied phenomena of bacteriolysis and of hæmolysis have not only demonstrated their fundamental similarity, but provided chemical explanations of the processes involved. Pfeiffer observed that the serum of immune animals caused agglutination only of the bacterial species used for immunization; he believed that for complete solution of the bacteria the mixture must be brought into the living body, and for this purpose he chose the peritoneum of the guinea-pig. Somewhat later Metchnikoff and Bordet discovered that the same effect could be produced *in vitro* by the addition to the immune serum of a small quantity of peritoneal exudate, or even of the fresh serum of an animal. From this experiment it could be inferred that for agglutination of bacteria certain bodies were required which were in the immune serum; but for solution still other substances contained in part in fresh serum were requisite. That this second substance might also be present in the original immune serum could now be shown; and it was also demonstrated that it is of a very labile nature and quickly disappears spontaneously. The addition of fresh serum or exudate restores it. It may be destroyed by raising the temperature of the fluid to 56° C.

A great advance in our knowledge of cytolysis was made when it was

[6] *Trans. College of Physicians of Philadelphia*, 1897, 3. s., xix, p. 105.

discovered that immunization to blood and body cells gives rise to the production of lysins. Just as in bacterial immunization lysins for the special bacteria employed are yielded, so also are evolved analogous substances for red and white blood cells, for epithelial cells, spermatozoa, etc. Indeed the number and variety of lysins that can be produced experimentally are limited only by the number and variety of animal cells available. Blood cells of one animal may be used to produce a lysin in the body of another animal of the same species—isolysin; in another species—heterolysin; and success has in rare instances followed the re-injection of withdrawn blood through which autolysins have been produced. Nor is the production of lysins the only result of the injection of cellular structures. Preceding the solution, clumping of the cells takes place from which it may be concluded that agglutinins are also formed.

The factors required for producing solution of cells are similar to those for causing solution of bacteria under like conditions. Only when the lytic serum is very fresh will solution be effected; the addition, however, of peritoneal exudate or fresh normal serum to immune serum which has lost the solvent property, suffices to restore it.

A consideration of the preceding facts shows that the agglutinating principle is distinct from the dissolving one. This consideration also indicates that more than one body is necessary to bring about solution either of bacteria or of animal cells.

An analysis of the phenomena suggests that at least two substances are requisite. One is stable and contained in the immune sera (whether for bacteria or animal cells); the other is labile, and while originally contained in the immune sera, it is lost spontaneously. This latter substance is a normal constituent of the lymph and blood plasma, for it can be restored by the addition of these fluids.

Experiments conducted in a very convincing way by Ehrlich and Morgenroth indicate: (1) that a special principle is concerned in agglutination—the so-called agglutinin; and (2) that two principles are concerned in lysis. These principles are different in origin. One—that which is stable—is the product of immunization, and, on account of certain combining properties possessed by it, they call it the 'intermediary body.'[7] The other is normally present in the body juices but

[7] Ehrlich has recently suggested the name 'ceptor,' in place of 'intermediary body.' According to the manner of action he distinguishes 'uniceptors' and 'amboceptors.' Bordet calls this body 'substance sensibilisatrice;' Metchnikoff 'fixator;' P. Mueller, 'copula.'

is easily destroyed by heat and tends to disappear spontaneously when the fluids are removed from the body. This latter principle, on account of the complemental nature of its action, they propose to call the 'complement.'[8]

There is conclusive experimental evidence that, although the intermediary body unites first with the cells—bacterial, blood cells, etc.—this substance by itself cannot bring about solution. But after the union of this intermediary body with the cells the complement is capable of being brought into action, through this intermediation, so that solution takes place. The union of intermediate body and cells is conceived to take place through certain combining (haptophore) groups present in the cells and in the intermediary substance; while the complement is linked through similar combining (haptophore) groups possessed by the intermediary body and itself. The intermediary body, therefore, carries two sets of combining or haptophore groups: one for the cells and the other for the complement (complementophilic group). The complement possesses in addition to such a corresponding haptophore group, another group which exhibits fermentative properties (zymotoxic or toxophore group), through the action of which solution of cells takes place.

This conception of lysis applies not solely to that produced by immunization, but the same factors are believed to be operative in the solution of blood cells or of bacteria by normal blood sera. Here also an intermediary body and a complement are brought into action; the only difference being that, in the one case, the intermediary body is produced through artificial immunization, and in the other, it is present normally, but whether because of some insidious and unperceived change, similar to but slighter than artificial immunization, is not known.

It would carry us too far afield to give in detail the elaborate views of Ehrlich and his co-workers as to the origin of the intermediary bodies. Suffice it to say that they are the products of immunization by bacterial or other cells, and are believed by them to be yielded by certain constituents of cellular protoplasm within the body, designated as 'lateral chains,' which through their haptophore groups are capable of combining with the haptophore groups of protoplasmic constituents of the bacteria or the cells used for the immunization. When dealing with the toxic constitution of venom more will be said concerning this aspect of the subject.

[8] This body is called 'alexin' by Bordet, and probably agrees in part with the body of the same name described by Buchner. Metchnikoff calls it 'cytase.'

VENOM-AGGLUTINATION.

For the study of agglutination all the available varieties of venom were employed. Several kinds of animal blood—from the dog, rabbit, guinea-pig, sheep, ox, pig, *Necturus*, and frog—were tested. Either the blood was defibrinated or the specimen consisted of the corpuscles separated by centrifugalization and washed six times, as a rule, in 0.8% normal saline solution (washed corpuscles). The different venoms showed slight differences only in the degree of agglutination, with the exceptions of the action on the blood of *Necturus* and the frog, which are not affected in weak solutions but show agglutination in stronger solutions (2%).

The usual method consisted in dissolving in normal saline solution dried venom in strengths ranging from 0.01% to 10%—the last that of *Crotalus adamanteus*. The phenomena of agglutination appear rapidly in favorable solutions, while in very weak solutions a delay of some minutes up to one hour may be noted. The corpuscles which come together thus slowly do not show the great modification of shape that is characteristic of those that fuse more completely and quickly. In a general way it may be said that the several varieties of dried venom with which we experimented gave, when employed in the strength of 0.5%, what are to be regarded as maximal agglutinations for mammalian corpuscles. Active agglutination still takes place in 0.2% solutions, while weaker ones either produce no change at all or show an imperfect fusion.

The morphological changes need not be described here as these have been fully dealt with by Mitchell and Stewart.[9]

The value of the use of washed corpuscles comes especially from the fact that the succession of lytic phenomena is eliminated. Agglutination, therefore, may be studied purely. For this purpose a 5% solution of the corpuscles in normal salt solution was employed. That complete agglutination has no effect upon subsequent solution (lysis) of the corpuscles will be shown when treating of the latter phenomena. On the other hand, distinct differences in susceptibility

[9] Loc. cit.

to agglutination have been observed. Of the mammalian blood thus far employed the red corpuscles of the rabbit may be said to be highly susceptible, while those of the guinea-pig, dog, sheep, swine, and ox were less responsive in about the order given.

The use of defibrinated blood permitted observation upon the succession of phenomena of agglutination and hæmolysis. In general it may be said that the first effect of the venom is the production of agglutination to be followed by solution after a variable interval, depending on the kind and strength of the venom and on the temperature. There are, however, notable exceptions in that the range of lytic activity of venoms is greater than that of the agglutinating property. Very weak solutions of venom which no longer cause agglutination may still be capable of producing solution.

Moreover, on account of the action of two sets of factors in defibrinated blood—one tending to produce agglutination, and the other solution of the red corpuscles—the degree of agglutination is here less marked than in the washed cells where no lysis occurs. This difference is explained by the fact that a part of the corpuscles go into solution before agglutination can take place; and hence the extent of precipitation and fusion varies inversely with the susceptibility to lysins. As a consequence, dog's corpuscles which are more easily hæmolysed by venom than any others of the animal bloods tested by us show the least degree of agglutination. The rapidity of agglutination in any case is not affected by ordinary temperatures. Hence a low temperature (0° C.) permits, even in defibrinated blood, the separation of the phenomena of lysis and those of agglutination. At this temperature defibrinated dog's blood behaves as do the washed corpuscles, the amount of precipitation being, therefore, greater than at the temperature of the room or of the thermostat.

The agglutinating power of venoms is destroyed by temperatures of 75° to 80° C. maintained for thirty minutes.

VENOM-HÆMOLYSIS.

Unless mention is especially made the same group of animals was employed in these studies as in the foregoing. The venom solutions

varied from 5% to 0.0001%, depending upon the source of the corpuscles and the variety of venom. The venoms differ in hæmolytic power as follows: cobra most active; water mocassin, copperhead, rattlesnake, in less degree in the order named. A similar variation in susceptibility to the reaction could be distinguished in the different mammalian bloods employed. Thus dog's blood was most quickly and easily hæmolysed and it responded to the greatest dilutions, while the corpuscles of the ox were the least susceptible. The intermediate animals were in about the following order: sheep, guinea-pig, pig, and rabbit. On the other hand, the blood of *Necturus* is acted upon slightly after longer periods, and frog's blood almost not at all after equally long periods.

The extent of variation in the response of the different bloods is considerable. Very strong solutions of venom (5%) are needed to cause hæmolysis of corpuscles of the ox, but such great strengths are without action upon rabbit's corpuscles, although they are still capable of producing rapid solution of dog's or sheep's corpuscles. Taken altogether solutions averaging 0.2 per cent of venom have proven the most favorable for bringing out the hæmolytic effect upon blood generally.

Defibrinated Blood.—In making the tests with defibrinated blood uniform mixtures were employed. In all experiments 5 per cent of blood was added to the venom solutions, and the mixtures were kept at temperatures varying from 36° to 37° C.

The differences in activity of venoms are shown by the following series in which minimal active solutions are given for several kinds of blood.

Dog's blood hæmolysed by solutions of *Crotalus* venom 0.001%; copperhead 0.0005%; water-mocassin 0.0002%; cobra 0.0001%

Sheep's blood hæmolysed by solutions of *Crotalus* venom 0.002%; copperhead 0.0005%.

Guinea-pig's blood hæmolysed by solutions of *Crotalus* venom 0.002%; copperhead 0.001%.

Swine's blood hæmolysed by solutions of *Crotalus* venom 0.002%; copperhead 0.001%.

Rabbit's blood hæmolysed by solutions of *Crotalus* venom 0.005%; copperhead 0.002%.

Rabbit's blood hæmolysed by solutions of water-mocassin venom 0.002%; cobra 0.001%.

Ox's blood hæmolysed by solutions of *Crotalus* venom 0.05%; copperhead 0.02%.

Effect of Heat upon Hæmolytic Power of Venoms.—Temperatures of 75° to 80° C. for thirty minutes have no effect upon the hæmolytic action of any kind of venom. From 90° to 96° C. *Crotalus* venom in solution suffers a moderate reduction in hæmolytic power, while the remaining venoms are entirely unaffected at these temperatures. After heating to 100 C. for fifteen minutes the dissolving power of cobra, mocassin, and copperhead venom in solution is slightly reduced.

Effect of Venoms upon Washed Blood-Corpuscles.—In no instance were the washed blood-corpuscles hæmolysed by venom. Agglutination occurs as already described. But if the separated serum is restored to each of the several kinds of blood-corpuscles treated with venom, lysis takes place.

Certain important differences may be noted. Thus if the quantity of serum added to rabbit's corpuscles exceeds the normal, quicker and more complete solution occurs than is noted in defibrinated blood. A similar but less striking action may be observed with the blood of the guinea-pig. This action depends upon the union first effected between the red corpuscles and the intermediary body of venom, the latter later combining with the introduced complement of the serum so that solution takes place. That this is the mechanism of the action the succeeding experiments prove. But before taking them up another set of phenomena must be briefly considered.

Rabbit's and guinea-pig's washed blood-corpuscles (hereafter termed washed corpuscles) are quickly dissolved by fresh dog's serum. Dog's corpuscles are but little acted upon by fresh rabbit's serum and not at all by guinea-pig's serum. Rabbit's and guinea-pig's sera are about equally hæmolytic for each other's corpuscles. *Necturus*' serum is highly hæmolytic for rabbit's, dog's, and guinea-pig's corpuscles. Frog's serum is less destructive for the mammalian cor-

puscles mentioned than that of *Necturus*, but is still very active. *Necturus*' serum and frog's serum are slightly and equally active on each other's corpuscles.

This dissolving action is not noted at freezing temperatures. If, therefore, washed corpuscles which have been treated with serum for thirty minutes at zero temperature are separated by centrifugalization or precipitation, the complement in the serum is unaffected, while the intermediary body will be found to have been removed from the serum by the corpuscles. In this way the complement for a particular species of corpuscles, free from the intermediary body, can be obtained. The addition of such complement-containing serum to venomized washed corpuscles of the same species brings about hæmolysis, while the addition of fresh washed corpuscles to the treated serum, from which the intermediary body for them has been removed, is unattended by solution.

The action of complements, freed from any intermediary body by this means, upon venomized corpuscles of different species has also been studied. The results are of interest. The procedure is as follows: Let us suppose that it is desired to test the effects of rabbit's serum upon venomized dog's corpuscles. The rabbit's serum is first treated with washed dog's corpuscles in the cold to withdraw all combining intermediary bodies; the clear serum having been separated is now added to the venomized corpuscles, when solution of a slow and limited nature occurs, thus showing that there exists in the dog's corpuscles a limited number of receptors capable when venomized of uniting with rabbit's complement.

Controls for these experiments were made in the following manner: Any serum treated with alien corpuscles at zero temperature and then separated from the corpuscles by centrifugalization has become inactive for this kind of fresh washed corpuscles (tested for dog's, guinea-pig's, rabbit's, and *Necturus*' corpuscles). However, if to the inactive mixture the same variety of serum heated to 58° C. is added (this serum containing intermediary body but without complement) solution takes place.

The degree of interaction of different species of sera minus inter-

mediary bodies upon different species of venomized washed corpuscles is shown by the following series, in which copperhead venom is used throughout. 1 cc. of 0.2% solution of venom is mixed with 0.05 cc. of washed corpuscles and 0.5 cc. of complement, with these results:

Dog's corpuscles and rabbit's complement = slow and imperfect hæmolysis.

Dog's corpuscles and guinea-pig's complement = slight hæmolysis, more marked than preceding.

Rabbit's corpuscles and dog's complement = rapid and imperfect hæmolysis.

Rabbit's corpuscles and guinea-pig's complement = weak and imperfect hæmolysis.

Guinea-pig's corpuscles and dog's complement = rapid and imperfect hæmolysis.

Guinea-pig's corpuscles and rabbit's complement = slow and imperfect hæmolysis.

Dog's corpuscles and *Necturus*' complement = slight and imperfect hæmolysis.

Guinea-pig's corpuscles and *Necturus*' complement = no action.

Rabbit's corpuscles and *Necturus*' complement = no action.

Venom solution treated with dog's, rabbit's, and guinea-pig's washed corpuscles in succession gives up to each a part of its intermediary bodies. No one kind of corpuscle is capable of fixing the entire content of intermediary bodies. The supernatant fluid probably contains still other intermediary bodies capable of fixation by still other corpuscles. If to the several kinds of venomized corpuscles here mentioned different complements are added, then, as shown in the foregoing series, lysis will or will not take place, depending on the nature of the complement employed; but so long as the complement is foreign to the corpuscles it never causes complete solution.

From these results the following conclusions are warranted: (1) Venom contains several or many intermediary bodies. (2) These bodies show specific affinities for certain complements. In addition to this there is evidence that the many susceptible corpuscles contain, besides specific haptophore groups for intermediary bodies, certain common haptophore groups, which are shared, perhaps, by all vulnerable corpuscles.

Combined Action of Venom and Ricin. Relation of Agglutination and Hæmolysis.—Agglutination produced by venom does not affect lysis. But on the other hand when lysis takes place quickly, agglutination may fail or may appear imperfectly. The principles causing the two phenomena are distinct in the manner of combination and in action. That the agglutinative and the lytic principles are different is now proven; and there is evidence that they act upon different constituents of the red cells. Thus, if ricin, a strong agglutinator, is permitted to act upon red corpuscles for periods under thirty minutes, then upon the addition of venom lysis ensues in about the average time and proceeds normally. If, however, the ricin has acted for two or more hours, then solution by venom still takes place, but the stroma of the corpuscles remains in the bottom of the test-tube as a white conglutinated mass. From this it appears that agglutination brings about a kind of coagulation of the stroma, from which, through the action of the hæmolysin in venom, hæmoglobin has been released. Ricin is without action upon venom itself, and conversely ricin is equally unaffected by venom.

VENOM-LEUCOLYSIS.

In the blood snake-venom causes destruction of the leucocytes as well as of the red cells. In order to ensure a more accurate study of its action upon the white blood-cells, these were obtained in larger quantities and without admixture of red cells by injecting positively chemotactic substances into the pleural and peritoneal cavities of the rabbit. For this purpose cultures of *B. megatherium* killed by heat were injected into the pleural cavity and sterile bouillon into the peritoneum.

From eighteen to twenty-four hours after the introduction of *B. megatherium* into the pleural cavity, fluid rich in leucocytes may be withdrawn by means of capillary tubes without sacrificing the animal. The same procedure employed twenty-four hours after the injection of bouillon yielded a fluid less rich in leucocytes, and consequently this second method was not extensively employed.

The leucocytes in the fluids thus obtained could be separated ac-

cording to size and granulation into lymphocytes (20 to 25% of the total leucocytes), finely granular, medium-sized cells (60%), larger non-granular cells (3%), still larger irregular and coarsely granular cells (4%), and others again somewhat smaller but showing very coarse granules (6%). The susceptibility to the destructive effects of venom varied somewhat for the different cells. Those of the largest size with coarse granules are most quickly affected; next to these come the finely granular varieties, the lymphocytes showing the least injury of all.

In studying the changes taking place in the leucocytes under the influence of venom a warm stage (37° C.) was used, the edges of the cover glasses having first been sealed with vaseline. The venom solutions varied from 10% to 0.002%. The weakest effective solution was that of cobra venom (0.002%), whereas in the case of the rattlesnake and of the mocassin, 0.002% and 0.005% respectively, caused definite changes.

Only the granular cells showed motility. Weak active solutions are without immediate effect on motion but begin to manifest an inhibiting action after about one hour, the controls being still motile at the end of two hours or longer. After the motility ceases, the cells in general, except the lymphocytes, show increased granulation due to the appearance of coarser and more numerous granules in the protoplasm, the nuclei coincidently becoming more distinct. After six hours the majority of the largest granular cells have already disintegrated, the nuclei having been liberated. After twenty-four hours most of the medium-sized granular cells have suffered disintegration, while the lymphocytes show but slight and inconspicuous changes. Stronger solutions, varying from 0.2% to 10%, cause instant cessation of motility and rapid agglutination without distinction of variety of cells. Within five to thirty minutes thereafter dissolution sets in, affecting first the largest, then medium-sized cells, and finally the small lymphocytes.

There are variations in the activities of the several venoms and in the completeness of solution of the cells. Rattlesnake venom is far less active than that of the cobra. Thus in 2% solutions cobra venom

causes complete solution in thirty minutes while that of the rattlesnake requires two hours to bring about the same result.

The effects upon washed leucocytes differ from those described in that venom solutions cause agglutination, but with the production of only very slight lysis.

Are the Hæmolysins (Erythrolysins) Identical with Leucolysins?—Copperhead venom (1 mg. in 4 cc. of normal saline) was treated with washed rabbit's red corpuscles at the thermostat temperature for thirty minutes, until the supernatant fluid after centrifugalization was without action upon defibrinated rabbit's blood. This solution when brought into contact with leucocytic fluid was without agglutinating action upon the cells while still causing their solution in about thirty minutes. On the other hand, the parallel experiment in which venom solution was treated with washed leucocytes yielded a fluid still active for defibrinated blood.

The conclusions from these experiments are as follows:

(1) Venom contains principles which are agglutinating and dissolving for white blood-corpuscles.

(2) The agglutinating principles may be identical for both white and red cells.

(3) The dissolving principle for leucocytes is distinct from that for red cells.

(4) In order that solution of venomized leucocytes shall occur a complement-containing fluid is required.

(5) The several varieties of white cells of the rabbit's blood show different susceptibilities to the action of venom.

VENOM-TOXICITY.

For the study of the toxic principles copperhead venom was chiefly employed. The animal selected for these experiments was the guinea-pig. The method of procedure was the following: We first determined for the particular sample of venom to be used the minimal lethal dose. This was found to be 0.3 mg. for a guinea-pig weighing from 250 to 300 grammes, death resulting within 24 hours. A dose of 0.6 mg. caused death in from two to three hours, and of 0.9 mg. in from 30 to 45 minutes.

Our special purpose was the determination of the existence of neutralizing substances for venom in the tissues of the body. The following tissues and organs were employed: brain, liver, spleen, kidney, voluntary muscle, adrenal gland, and blood. For this purpose the tissues were first washed in tepid, sterile, normal saline and a weighed quantity (2 grammes) was taken. This was triturated in a sterile mortar and mixed in test tubes with three times the minimal lethal dose (M. L. D.) of venom.

The mixture was now placed in the thermostat where it remained for one hour. It was then centrifugalized and the supernatant fluid was injected into the guinea-pigs. In the course of the experiment the original volume of the venom-solution suffered a loss amounting on an average to one-third of the whole volume. There should, therefore, after this subtraction remain behind at least twice the minimal lethal dose (2 × M. L. D.).

In the case of the blood, washed corpuscles were employed in excess, the other steps remaining the same as in previous experiments.

The results of these experiments are as follows:

Control	Dead in	45 minutes.
Brain	"	19 hours.
Blood	"	3 hours and 50 minutes.
Adrenals	"	2 hours and 35 minutes.
Spleen	"	2 hours and 10 minutes.
Liver	"	1 hour and 30 minutes.
Kidney	"	1 hour and 55 minutes.
Muscles	"	1 hour and 30 minutes.

In the next experiment 2 M. L. D. of venom were employed. The control died in five hours. The animals receiving the venom solution treated with the organs, etc., reacted as follows:

Brain	Survived.	
Blood	Dead in	28 hours.
Liver	"	19 hours.

Relation of Neurotoxic to Hæmolytic Principle.—That these two principles are distinct is rendered probable by the effects of washed red-corpuscles and of brain tissue respectively upon the toxicity of

venom. Blood-corpuscles remove little or perhaps none of the toxic constituent that brain cells do away with *in toto*. The proof of difference can, however, be brought in another way: Four M. L. D. of venom were treated with an excess of red corpuscles and the supernatant fluid was injected into a guinea-pig; death ensued in 30 minutes. The same quantity of venom having been treated with four grammes of brain emulsion, the supernatant fluid injected into a guinea-pig caused death in 48 hours. The experiments in which 9 M. L. D. of venom were used resulted, (1) in the case of the blood in death in 25 minutes; and (2) in the case of the brain (7 grammes of brain having been employed) in 25 hours.

The supernatant fluid from the brain emulsion was strongly agglutinating and hæmolytic for defibrinated blood, while that from the washed corpuscles had lost all these properties. The supernatant fluid from the brain emulsion, when treated with an excess of washed corpuscles, re-centrifugalized and the fluid then injected, is non-toxic for guinea-pigs.

These experiments show: (1) that the neurotoxic and the hæmolytic principles are physiologically distinct; (2) that while the chief toxic constituent unites with the nerve cells, in multiple M. L. D. from which the neurotoxic principle has been removed a quantity of hæmolysin may be contained sufficient to bring about fatal intoxication.

These results are in keeping with the views expressed by Ehrlich and supported by Wassermann and Takaki's experiments on the fixative power of cells for certain groups of toxic substances. They tend, therefore, to support the hypothetical considerations of Ehrlich on which he bases his well-known lateral-chain theory of immunity. Expressed in the terms of this hypothesis brain cells may be said to contain the receptors for the neurotoxic constituent of venom, whereas blood cells furnish the receptors for the hæmolytic principle; these receptors are distinct and specific, and are not contained to any considerable amount, and perhaps not at all, in the liver and kidney cells and, if at all, in small quantity only in adrenal cells. Walter Myers found that the adrenal cortex possessed a very feeble combining power

for cobra venom, most marked in mammals (sheep) in which the cortex of the organ is well developed. He also observed little effect from the adrenal of the guinea-pig.

EFFECTS OF VENOM UPON BACTERICIDAL PROPERTIES OF BLOOD SERUM.[10]

The animals employed were the dog, rabbit, and *Necturus;* the venoms belonged to the cobra, moccasin, copperhead, and rattlesnake, and the bacteria were *B. typhi*, *B. coli*, and *B. anthracis*. The method consisted in (1) introducing venom into the animal and drawing the blood from the femoral artery into sterile Nuttall bulbs; (2) permitting the blood from the normal animals to enter Nuttall bulbs in which the venom solution was contained: (3) admixture of the venom in sterile solution (heated for 4 days to 56°-60° C.) with separated serum.

The bactericidal effects of the normal sera were first established. Rabbit's serum is highly destructive for *B. typhi* and *B. anthracis* and least for *B. coli*. Dog's serum is highly destructive for *B. typhi*. *Necturus*' serum is also very destructive to *B. typhi* and *B. coli*. It is without marked effect on *B. anthracis*.

Serum Venomized in vivo.—Cobra venom was most active. Blood from rabbits which had received 10 mg., taken 57 minutes after the injection, showed great loss of bactericidal properties.

Experiment LXXI.—1 cc. venomized serum employed.

	B. anthracis	*B. coli*	*B. typhi*
Immediate	1841	4254	7152
After 1 hour	767	3125	4130
" 3 hours	2488	13460	4320
" 6 "	Innumerable	Innumerable	17280
" 24 "	"	"	Innumerable

The controls for this experiment showed complete destruction of all bacteria, or, as in a few experiments, of all except *B. coli*, which

[10] In order to determine whether any effect is produced on the growth of bacteria by the presence of venom in culture media, varying small quantities of venom were added to nutrient agar-agar. The bacteria—*B. anthracis*, *B. coli*, *and B. typhi*—grown upon these tubes underwent rapid involutions and exhibited marked plasmolysis, as compared with control tubes of the same organism.

showed considerable diminution until after six hours when increase began.

Experiment LXX.—30 mg. rattlesnake venom injected; blood taken after 45 minutes. 1 cc. venomized serum employed.

	B. anthracis	*B. coli*	*B. typhi*
Immediate	838	9940	5750
1 hour	756	6240	3654
3 hours	2930	Increase	6219
6 "	Increase	Innumerable	About 10000
24 "	Innumerable	Innumerable	Innumerable

Blood mixed with Venom in vitro.—In this series rabbits only were employed. The venom solutions were placed in Nuttall's bulbs and the blood from the femoral artery was permitted to stream into them. In each experiment 6 mg. of venom were mixed with 20 to 30 cc. of blood. Coagulation was very slow or completely inhibited and the serum was obtained when necessary by centrifugalization. It invariably contained hæmoglobin.

Experiment LXXIII.—1 cc. of venomized serum employed.

	B. anthracis	*B. coli*	*B. typhi*
Immediate	1644	4580	4085
1 hour	2080	10760	4870
3 hours	18930	149740	24730
6 "	Innumerable	Innumerable	Innumerable

Experiment LXXII.—1 cc. venomized serum employed.

	B. anthracis	*B. coli*	*B. typhi*
Immediate	736	3720	1275
1 hour	407	2340	920
3 hours	860	22210	8720
6 "	5220	Innumerable	Innumerable
24 "	Innumerable		

This series of experiments may be open to the criticism that the increased nutritive value of the serum because of the hæmoglobin present may have been the cause of the effects noted; as a control,

therefore, peptone was added to the serum in the proportion of 6 mg. of peptone to 20 cc. of serum.

Experiment LXXX.—Peptone added to rabbit's blood: 1 cc. employed.

	B. anthracis	*B. coli*	*B. typhi*
Immediate	1043	5120	7430
1 hour	193	2240	1534
3 hours	87	578	71
6 "	22	520	262
24 "	0	Innumerable	About 20000

From this experiment it follows that improvement in nutritive value reduces bactericidal effect but in far less amount than is noted in the parallel case of venom.

That the nutritive change is unimportant is shown by the first experiments, in which the poisoning was done *in vivo* and also by those to follow in which venom was added directly to the separated serum.

Experiment LXXXV.—Rabbit's serum 1 cc. with rattlesnake venom 1 mg.

	B. anthracis	*B. coli*	*B. typhi*
Immediate	745	3990	5430
1 hour	594	4667	3136
3 hours	4486	12120	43430
6 "	About 100000	Innumerable	About 200000
24 "	Innumerable		Innumerable

Experiment LXXXII.—Dog's serum 1 cc. with copperhead venom.

	Venom 6 mg. *B. typhi*	Venom 1 mg. *B. typhi*	Control; norm. ser. *B. typhi*
Immediate	8860	3572	5808
1 hour	21120	6525	584
3 hours	65250	14950	184
6 "	Innumerable	Innumerable	92
24 "			0

In order to determine the least quantity of venom required to remove the bactericidal properties of the serum varying quantities of copperhead venom were employed. Dog's serum was chosen with *B. typhi*. In each case 1 cc. of serum was used.

Experiment LXXXVII (a).—1 cc. dog's serum and varying amounts of copperhead venom.

	Venom 1/2 mg.	1/5 mg.	1/10 mg.	1/20 mg.	1/50 mg.
Immediate	5970	3070	4290	4940	3350
1 hour	6240	3960	1830	2620	920
3 hours	12810	10000	6730	1350	593
6 "	Innumerable	100000	13140	172	15
24 "		Innumerable	Innumerable	About 10000	0

From this it may be concluded that the specimen of venom employed by us destroys the bactericidal properties of dog's serum when added in the proportion of 1/20 mg. of venom to 1 cc. of serum; and that 1/50 mg. in the same quantity of serum is practically without action.

In view of these positive results the partial inaction of venom upon *Necturus* serum is both remarkable and important.

Experiment CII.—1 cc. *Necturus* serum employed.

	B. anthracis	*B. coli*	*B. typhi*
Immediate	4215	5127	8350
1 hour	6950	453	233
3 hours	18340	84	12
6 "	50170	4	0
24 "	Innumerable	0	

Experiment CIII.—Same as above and copperhead venom 6 mg.

	B. coli	*B. typhi*
Immediate	5237	3846
1 hour	615	394
3 hours	95	11
6 "	180	2
24 "	2950	3105

Control: *Necturus* serum 1 cc. + peptone 6 mg.

	B. coli	*B. typhi*
Immediate	9270	4530
1 hour	3810	577
3 hours	632	92
6 "	78	2
24 "	1850	927

Experiment CIV.—*Necturus* serum 1 cc. + copperhead venom 1 mg.

	B. coli	*B. typhi*
Immediate	6240	7360
1 hour	1170	833
3 hours	583	96
6 "	25	13
24 "	0	0

It may be remarked that *Necturus* is highly refractory to venom. An animal weighing 250 grammes received without effect 0.05 grm. venom, equivalent to 160 M. L. D. for the guinea-pig.

The effect of heat upon venom in relation to its action upon the bactericidal properties is of interest. For this purpose cobra, rattlesnake, moccasin, and copperhead venoms were studied. Temperatures varying from 75° to 90° C. were employed, and the heated venoms were mixed with the streaming blood in Nuttall's bulbs and with the separated serum. The venom was kept at the lower temperature (75°) for 30 and the higher (90°) for 15 minutes.

The heated venom acts just as the unheated except in the case of rattlesnake venom, the effect of which is somewhat diminished at the higher temperature, 90° C.

The Mechanism of the Action of Venom upon Serum.—That the bactericidal action of serum depends upon the intermediary body and complement seems established. That the influence of venom upon this property does not depend upon changes in the nutritive value of the serum the foregoing experiments prove conclusively. It is therefore possible that venom acts injuriously upon the intermediary body or the complement or upon both bodies at the same time. The complement is destroyed by heating serum to 56°-58° C.—a temperature which does not affect the intermediary body.

Experiment XCVIII.—To test effect of venom on the intermediary body.

(1) Copperhead venom 1/20 mg.; rabbit's serum 1 cc., and rabbit's serum heated to 58° C., 1 cc. (2) Control: rabbit's serum heated to 56° C.

	(1) *B. typhi*	(2) *B. typhi*
Immediate	4990	5320
1 hour	5800	7800
3 hours	18840	12400
6 "	Innumerable	Innumerable

Experiment XCIX.—To test effect on the intermediary body.

(1) Copperhead venom 1/10 mg.; dog's serum 1 cc.; dog's serum heated to 56°, 1 cc. (2) Control; serum heated to 56° C.

	(1) *B. typhi*	(2) *B. typhi*
Immediate	2270	3440
1 hour	2680	3950
3 hours	11950	12800
6 "	Innumerable	Innumerable

From these experiments the conclusion can be drawn that venom is without action upon the intermediary body contained in dog's and rabbit's serum.

The next experiment was to determine whether any action was exerted by venom upon the complements of these sera. For the purpose of obtaining the serum-complement free from the intermediary body, the rabbit was treated with dog's serum heated to 56° C. In this way the anti-intermediary-body was obtained, which, when heated to 56° C. (to remove rabbit's complement) and added to fresh dog's serum neutralized the action of the latter upon rabbit's corpuscles. From this it could be concluded that the intermediary body of the dog's serum was neutralized by the anti-intermediary-body contained in the immunized rabbit's serum, leaving behind the pure dog's complement in the fluid.

Experiment XCIX (a).—Action on complement.

(1) Fresh dog's serum 1 cc., and copperhead venom 1/10 mg., and dog's complement 1 cc.[11] (2) Control, dog's serum 1 cc., and 1/10 mg. venom.

[11] To obtain these complements fresh dog's or rabbit's serum was treated with rabbit's or guinea-pig's serum containing the anti-intermediary-body which was heated

	(1) *B. typhi*	(2) *B. typhi*
Immediate	5270	4360
1 hour	930	5980
3 hours	28	25410
6 "	15	Innumerable
24 "	0	

A similar experiment in which anti-intermediary-body for rabbit's serum was produced in the guinea-pig gave practically identical results except that when 1/10 mg. of venom was employed the neutralizing effect of this quantity on the complement was also exerted upon the second quantity of complement added.

Experiment XCVIII (a).—Copperhead venom; fresh rabbit's serum 1 cc., and rabbit's complement 1 cc.

	B. typhi 1/10 mg. venom	*B. typhi* 1/20 mg. venom
Immediate	4590	3280
1 hour	3740	1360
3 hours	1850	730
6 "	4900	110
24 "	Innumerable	0

From the experiments under the present heading the following conclusions are warranted:

(1) All venoms when used in suitable quantities destroy the bactericidal properties of many normal blood sera.

(2) The manner of this destruction consists in the fixation of the serum-complements by the venoms.

(3) Venoms have no action upon the intermediary bodies of serum.

(4) If the venom is incapable of uniting with the serum-complements (*Necturus*) then the original bactericidal properties remain unaffected by the presence of the venom.

EFFECTS OF ANTIVENIN ON HÆMOLYSIS AND BACTERIOLYSIS.

Through the kindness of Dr. McFarland we secured a small vial of Calmette's antivenin. This was used to test the restraining action

upon venom hæmolysis and venom anti-bacteriolysis. The antivenin was first proven to be non-hæmolytic for rabbit's corpuscles and to improve slightly the nutritive value of fresh rabbit's serum.

Erythrolysis by cobra venom on rabbit's corpuscles is prevented if neutralization by antivenin is effected. Thus 2 mg. of venom + 1 cc. of antivenin is still lytic although action is retarded; 1.5 mg. of venom + 1 cc. of antivenin caused slight hæmolysis after 24 hours, while 1 mg. + 1 cc. was without action.

In the case of rattlesnake venom 1 cc. of antivenin neutralized 3 mg. of the poison.

Leucolysis was affected in approximately the same degree as in the case of erythrolysis.

The effect on bacteriolysis is equally marked. When cobra or rattlesnake venom is treated with a neutralizing quantity of antivenin and fresh serum is added the resulting fluid behaves in a manner similar to that of the control mixture of normal fresh serum and antivenin.

Antivenin, therefore, neutralizes venom and removes both the hæmolytic and the anti-bacteriolytic actions.

ON A COCCIDIUM (*KLOSSIELLA MURIS*, GEN. ET SPEC. NOV.) PARASITIC IN THE RENAL EPITHELIUM OF THE MOUSE.

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Plates XXI–XXIII.

In 1889 one of us[1] published a preliminary description of a polysporous coccidium found in the epithelium of the convoluted tubules of the mouse's kidney. Several stages of the parasite were seen but the scantiness of the material left many gaps in the life-cycle. During investigations recently made to determine the mode of transmission of the Sarcosporidium of the mouse one of us[2] found a considerable number of gray mice, caught in the animal room connected with this laboratory, whose kidneys were abundantly invaded by this coccidium. This favorable opportunity of examining more thoroughly into the life-history of this sporozoon was utilized, and, as a result, we are able to add materially to the knowledge of this cell parasite. In the following pages some details given in the first paper will be repeated to avoid obscurity in the descriptions as well as to aid those to whom the first paper is inaccessible.

Thus far we have found only adult mice infected. The invaded kidneys are a trifle enlarged and the surface is faintly uneven. The most characteristic feature is a very delicate mottling of the whole surface with minute, barely visible, grayish specks. This appearance of the kidneys may be considered almost diagnostic of the presence of the parasite.

[1] Smith. *Journal of Comparative Medicine and Surgery*, 1889, x, p. 211.

[2] Smith. *Journal of Experimental Medicine*, 1901, vi, p. 1.

METHODS.

To study the parasite in the fresh state a bit of the cortex is teased in physiological salt solution on a slide and gently spread by pressure on the cover-glass. This simple procedure enables us to gain an insight into that portion of the life-cycle of *Klossiella* through which it passes in the renal tubules, for, as a rule, all stages are present in the same heavily-infected kidney. Even the nuclei can be made visible by treatment with aceto-methyl green.[3] Schneider's aceto-carmine has been found less satisfactory as a nuclear stain for fresh material. Kidneys intended for sectioning were halved lengthwise, fixed and hardened in Zenker's fluid for 24 hours, washed in running water for the same period, and then passed through 50 and 70 to 95 per cent alcohol. Other fixing agents were tried (Flemming's fluid, aceto-sublimate, and picro-acetic), but they possessed no advantages over Zenker's fluid. The tissue was embedded in paraffine, melting at 51°-53° C., and cut in ribbons. Sections as thick as 10-15 μ were occasionally useful, as each section included more of the parasite, which, owing to its minuteness, is not easily studied in serial sections.

The most useful stains have been ferric-alum hæmatoxylin and Mayer's hæmatate of ammonia (hæmalum), the latter used alone or followed by Van Gieson's picro-acid fuchsin. The latter, however, can only be used after prolonged staining with the hæmalum, otherwise it will completely mask the nuclear stain. Fine results are obtained by staining with eosin followed by Unna's polychrome methylene blue. Ferric-alum hæmatoxylin gives by far the clearest and sharpest picture of any stain, but certain delicate differentiations of the cytoplasm are best brought out by prolonged staining (24 hours or longer) with hæmalum followed by picro-acid fuchsin.

THE DEVELOPMENTAL STAGES OF *KLOSSIELLA MURIS*.

An adequate description of the various stages found in the kidney will necessitate a certain order beginning with the earliest stage we have seen. Before proceeding to such a detailed account it may be well to summarize very briefly the life-cycle as we now know it.

[3] Glacial acetic, 5 pts., distilled water 95 pts., methyl green to saturation.

The youngest parasite is a minute nucleated cell (sporont) found within the cytoplasm of the epithelial cells of the convoluted tubules. This cell enlarges, the nucleus divides into 12 or more daughter-nuclei which are next found near the now slightly-lobed or mammillated periphery of the parasite (mother-sporoblast). The cytoplasm now separates into 12 or more spheres with each nucleus as a centre (sporoblast stage). These spheres secrete a membrane (spore stage), and the contents of each spore break up into about 30 nucleated, falciform bodies (sporozoites). Each parasite thus produces in the kidney at least 360 individuals. In severe infections the convoluted tubules are frequently found blocked with these spores. The sporozoites are not set free in the kidneys, as intact spores are found in the bladder. The further life-history of this parasite remains conjectural, although it is highly probable that the spores enter the body with the food and that the sporozoites are set free in the digestive tract.

To this cycle must be added another body of different morphology, which is parasitic in the epithelium of Bowman's capsule and whose significance will be discussed later on.

A. THE PARASITE OF THE CONVOLUTED TUBULES (SPOROGONIC CYCLE).

1. *The earliest stage (sporont).*—In fresh teased tissue the invaded cells frequently become detached and in these the sporonts are best studied. The host cell is enlarged, pear-shaped, and the parasite lies within it, not in contact with the cytoplasm, but in a vacuole (Plate XXII, Fig. 3). Not infrequently 2 sporonts are contained in the same cell; each then lies within its own vacuole (Plate XXII, Fig. 5). The sporont measures 8 μ to 11.5 μ in diameter.

A very striking feature of the sporont is the storage of reserve food material in the form of spherical, highly refractive granules, to the number of 10 to 20 (Plate XXII, Figs. 3 and 4). These are usually accompanied by smaller granules of the same optical appearance and chemical properties (Plate XXII, Fig. 4). The large granules measure 1.5 μ in diameter, the smaller, 1 μ or less. The former disappear at or towards the close of the sporont stage: the latter per-

sist and even increase in number long after the daughter-sporoblasts are formed (Plate XXIII, Fig. 14), but there is no indication that the larger granules break up to form the small ones. The granules of both sorts are doubtless of a proteid nature and come under the head of "plastin granules" described by various students of the Sporozoa. They are highly resistant toward all the chemical agents with which we have tested them. It is in fact possible with strong mineral acids and alkalies to dissolve all the rest of the cell and still leave the granules intact. The large granules stain a vivid yellowish-green with aceto-methyl green; the smaller granules remain unstained. Schneider's aceto-carmine fails to stain either kind of granule. Iodine in a solution of potassium iodide gives them a yellowish tint, which remains unchanged on the addition of dilute sulphuric acid. They are not blackened by osmic acid, and are not dissolved by absolute alcohol. We conclude that they are neither of a starchy nor of a fatty nature but a highly resistant proteid, such as is known to occur widely among Sporozoa.[4]

In fixed and stained preparations the shrinkage has usually obliterated the vacuole in which the sporont lies. The latter appears as if embedded in the cytoplasm of the host (Plate XXII, Figs. 5 and 7). Concerning the mode of invasion of the host cell by the sporozoite we are unable to give any facts. The parasite, when first seen, was already spherical in form and 7 μ in diameter. At this stage the invaded cells are considerably increased in size and have assumed the characteristic flask shape (Plate XXII, Fig. 7), with the parasite near the broad distal end of the flask. As the parasite grows the host cell must continue to grow. It protrudes far into the lumen of the tubule, almost occluding it, and it may even cause a local enlargement of the lumen (Plate XXII, Fig. 7). The peduncle by which the cell maintains its attachment to the basement membrane and through which it derives nourishment becomes more and more slender and assumes a very characteristic granular appearance (Plate XXII, Fig. 7, and Plate XXIII, Fig. 13). This portion stains darkly with hæmatoxylin

[4] Bütschli, Protozoa. Bronn's Thierreich, I, Abth. 1, p. 517, Leipzig and Heidelberg, 1882. Wasielewski, Sporozoenkunde, 1896, p. 51.

and picro-acid fuchsin. The cell nucleus becomes more and more flattened, owing to the pressure of the parasite, and is displaced to one side (Plate XXII, Figs. 7, 10, 11, and Plate XXIII, Figs. 13, 14). Sometimes it is moulded cup-like upon the surface of the sporont (Plate XXII, Fig 7, infected cell on the right); sometimes it appears shrunken or entirely flattened by the pressure of the sporont (Plate XXII, Fig. 7, infected cell on the left).

The nucleus of the young sporont is generally eccentric in position (Plate XXII, Figs. 3, 7) and contains one or two highly-refractive, deeply-staining karyosomes. Usually at this stage and later a clear, non-staining area (karyolymph) surrounds the karyosome and the chromatic mass within or on the edge of which the karyosome lies. The nucleus may undergo a precocious division and the daughter-nuclei distribute themselves near the periphery of the sporont (Plate XXII, Fig. 5). This arrangement is seen much more clearly in the mother-sporoblast stage (Plate XXII, Figs. 6 and 7), and is obviously the precursor of the formation of daughter-sporoblasts.

2. *The formation of spores (stage of mother-sporoblast and sporoblast).*—In the mother-sporoblast stage the parasite has increased much in size, measuring 40 μ in diameter in the fresh condition. At this stage there are sometimes 2 to 4 karyosomes in each nucleus, indicating a precocious segmentation of chromatic material preparatory to the formation of sporozoites (Plate XXII, Figs. 6 and 7).

The nucleus is now in the most favorable condition for study, but on account of its minuteness we have learned very little about it. Figs. 8 and 9 (Plate XXII) represent the usual conditions met with. The nucleus may be defined as a vesicle filled with clear fluid within which are the chromatin mass and one or sometimes two karyosomes. Sometimes karyosomes only are seen (Plate XXII, Fig. 9). When there are two, one is usually much smaller than the other, and stains darker. Not the slightest indication of mitotic phenomena has been seen. The vesicular structure of the nucleus is not retained apparently beyond the mother-sporoblast stage. In all later stages we have found merely one or more round, intensely-staining bodies to all appearances identical with the karyosomes of earlier stages.

The mother-sporoblast stage is marked by a migration of the nuclei to the periphery. They are usually spaced more evenly than Fig. 6 (Plate XXII) would seem to indicate. At this stage there are often 2 to 4 karyosomes in each nucleus (Plate XXII, Figs. 6 and 7). The cytoplasm surrounding each nucleus begins to be segmented off from the rest and forms a boss on the surface of the mother-sporoblast (Plate XXII, Fig. 10). For a brief period the daughter-sporoblasts are arranged in the form of a rosette (Fig. 1, lower left-hand corner). This is due to the centrifugal mode of development of the daughter-sporoblasts, as clearly shown in the figures just mentioned. They appear to bud out from the central mass, now entirely devoid of nuclei, and then rapidly to resorb the greater portion of the mass in question while their peduncles of attachment become reduced. One or two residual masses of angular form are often seen at a later stage, wedged in among the brood of daughter-sporoblasts. In other cases there appears to be no restiform body and our conclusion is that it has been entirely resorbed by the daughter-sporoblasts. The latter, to the number of 6 to 14, rarely more, are soon entirely separate and free within the vacuole (Plate XXIII, Figs. 13 and 14). They are nearly spherical in shape and average 12 μ in diameter, although smaller and possibly abortive ones are often found in the vacuole or cyst.

At this stage the host cell still maintains its attachment to the basement membrane by means of the much-attenuated peduncle. It nearly occludes the lumen of the tubule (Plate XXIII, Fig. 13). Its cytoplasm has been reduced to a mere shell enclosing the vacuole. The nucleus still persists.

3. *The formation of sporozoites.*—The nucleus of each daughter-sporoblast undergoes repeated divisions and these nuclear divisions are foreshadowed by divisions of the karyosomes at a stage considerably in advance of the formation of the sporoblasts themselves. These precocious divisions as well as those which come after the formation of the daughter-sporoblasts (Plate XXIII, Fig. 15) are preparatory to the elaboration of the sporozoites. These, to the number of 30 to 35, are so packed in the spore that most of them lie with their

long axes in one direction. The short axis of the spore is parallel to the long axis of the sporozoites (Plate XXIII, Figs. 16 and 17). In the fresh condition a ripe spore averages 16 μ by 13 μ. A transverse optical section (Plate XXIII, Fig. 18) shows that the sporozoites occupy very nearly all the space within the cyst. At an earlier stage than that represented in Fig. 18 a restiform body is seen within the spore. Before the spores are fully formed they may be seen attached to this body by one extremity (Plate XXIII, Fig. 17). It is probably entirely resorbed by the sporozoites.

The sporozoites are rather strongly curved and are blunt at both ends (Plate XXIII, Fig. 19). They measure 7 μ in a straight line from tip to tip and 3 μ in thickness. The nucleus is located midway of the length and is frequently elongated (Plate XXIII, Fig. 16). In the fresh state the sporozoite exhibits a number of granules, one of larger size than the rest often being seen at one extremity (Plate XXIII, Fig. 19). The living spores are always translucent enough to enable one to see the sporozoites within. This is not invariably true of spores in sectional material stained with ferric-alum hæmatoxylin. This stain is readily extracted from the cyst-walls of a certain number of the spores, but is retained by others to such a degree that they appear perfectly black (Plate XXI, Fig. 2 and Plate XXIII, Fig. 16). This difference we believe due to a slight increase in thickness or imperviousness of the cyst wall.

The spore membrane appears to be entirely structureless. In optical section, even with very high powers, it appears merely as a sharp line. Strong artificial digesting fluids (peptic and pancreatic) do not entirely dissolve it. It is not difficult to rupture it by cover-glass pressure and set free the sporozoites.

B. THE GLOMERULAR PARASITE.

In many of the glomeruli of certain heavily-infected kidneys we have seen a parasite of considerably larger dimensions and irregular lobate form (Plate XXI, Fig. 1, lower right corner, and Plate XXIII, Fig. 20). Its habitat is the thin epithelium—usually the visceral layer—of Bowman's capsule. It encroaches inward strongly on the glomerulus.

Only a few stages in the development have been seen. That represented in Fig. 20 (Plate XXIII) is perhaps the commonest. Every gradation between this and the later stage shown in Fig. 21 has been found, but earlier stages are extremely rare. After prolonged search we have found only one example of what corresponds to the very young or sporont stage of the tubule parasite. This had a large, oval, strongly-stained nucleus with a single karyosome. The parasite was embedded by a little more than half its diameter in a much-thickened region of Bowman's capsule close to the neck. As we have seen no intermediate stages between this and that shown in Fig. 20, we cannot positively assert that these two are genetically connected. Between the stages represented in Figs. 20 and 21 every intermediate condition has been seen. Masses of protoplasm are segmented off around each one of the numerous nuclei and each mass becomes eventually transformed into a falciform body. These are somewhat variable in size and shape, as shown in Fig. 21. On account of this variability it is difficult to state their dimensions. Perhaps 7 μ by 2 μ is a fair average.

How shall the glomerular body be interpreted? While we have failed to prove even that it belongs to the same species as the parasite in the convoluted tubules, there is very strong presumptive evidence that it does, inasmuch as we have not found it in any kidney not infected with *Klossiella muris*. On the other hand, we have failed to find it in certain lightly-infected kidneys.

The glomerular parasite may be brought within the cycle of *Klossiella muris* in one of two distinct rôles. In the first place, it may represent the schizogonic or Eimerian cycle of the species, and as such may antedate the whole sporogonic cycle of the tubules. If this be true the falciform bodies shown in Fig. 21 represent the merozoites, and the infection of the tubular epithelium is due entirely to the passage of these with the secretion along the tubule until a suitable host cell is found, into which the merozoite penetrates.

Another interpretation would regard this as the male element (mother-microgametocyte), in which the falciform bodies represent the microgametocytes and from the latter the microgametes may

arise somewhat as described by Siedlecki[5] for *Adelea ovata*, i. e. after the microgametocyte has reached the macrogamete. In one instance we have seen a falciform, nucleated body adherent to a young sporont, and in two or three instances, in fresh material, a granular-looking body in the same position. As we have not seen any flagellated bodies or the penetration of a nucleus into the female element, we lack the necessary evidence that the adherent body in question is actually the male element.

c. The probable life-cycle of *Klossiella muris* according to the preceding investigations.

We have suggested two quite divergent interpretations of the glomerular parasite neither of which is in conflict with our present knowledge of Coccidia. If we interpret this body as the male element its position in the capsular epithelium is almost a necessary one for it to occupy in order that the microgametocytes may reach the tubular epithelium. If we accept the interpretation that the glomerular parasite represents the asexual or so-called Eimerian cycle which furnishes the means for an internal multiplication, or, pathologically speaking, an auto-infection of limited extent, the position of the body in the glomerulus is still a prerequisite for the success of the next stage, the invasion of the tubular epithelium by the resulting merozoites. The final interpretation probably will not be made until analogous forms in other species of coccidia shall have been discovered in which the significance of the two stages may be subjected to demonstration. This species seems to be the first one in which two forms, genetically related, occupy epithelial cells of somewhat different morphological and physiological characters.

The spores, as already stated, pass out in the urine and are most likely taken in by mice in the food and water. Direct infection is inferred from the large percentage of infected mice which are found in cages where numbers have been living together for several months at least. This observation frequently made by one of us in the study

[5] *Annales de l'Institut Pasteur*, 1899, xiii, p. 169.

of sarcosporidiosis led to a number of feeding experiments with infected kidneys. The method of feeding and of keeping the mice under observation was the same as that used in the study of *Sarcocystis muris*, to which the reader is referred for details.[6] Only kidneys containing spores with fully-developed sporozoites were used. These were finely divided and mixed with crumbs of bread softened in physiological salt solution. The dish containing this food was placed in the jar with the mouse or mice to be infected. The limited number of experiments furnished no conclusive evidence. Only one deserves mention.

A mouse fed December 18, 1900, was killed February 16, 1901. The kidneys, carefully examined fresh and in sections, contained young stages only. In the spontaneous cases early and advanced stages occur together, suggesting repeated infection. In this the occurrence of only one stage is what we should expect from a single infection.

The falciform bodies (sporozoites) set free in the digestive tract probably bore their way into the circulation which carries them into all parts of the body. Their minute size permits them to enter the smallest capillaries. In the kidneys they leave the capillaries in the glomerulus to invade the capsular epithelium and the epithelium of the convoluted tubules. In all other organs they are probably suppressed.

PATHOGENESIS.

The presence of cell-parasites like the one under consideration is evidently not indifferent to the organ in which they multiply and mature; and it remains for us to consider very briefly what injuries may be inflicted and what processes induced.

The youngest stage of the parasite (sporont) leads to the formation of a vacuole in the epithelial cell which increases with the growth of the parasite. Its very attenuated walls may still be seen after the formation of the sporoblasts. As the sporont enlarges into the mother-sporoblast and from this about 12 spores are segmented off, each larger than the original sporont as first seen within the cell, the

[6] *Journal of Experimental Medicine*, 1901, vi, p. 1.

tubule within which the parasite is lodged becomes compressed, especially when a number of the parasites have matured close together. The compression causes the adjacent epithelium to disappear, and the tubule looks like an elongated contorted bag filled with spores but largely devoid of epithelium.

No so-called inflammatory reaction can be associated with these stages. The process is a mechanical one, leading to cell destruction. There is, however, another phenomenon associated with extensive invasion of the kidneys. These organs show over the entire surface a large number of minute grayish spots, already referred to. These minute spots represent foci of necrobiotic changes in the cortex, followed by marked cell proliferation in the same areas. The less advanced foci are largely made up of the tubular epithelium of the area which has become, as it were, fused into a mass on account of the obliteration of the lumina of the tubules. In later stages the original tissue becomes gradually obscured by the active proliferation of the intertubular tissue, giving rise to a dense collection of cell nuclei with scant cytoplasm. These foci resemble early foci in tuberculosis when giant cells are absent. The cells themselves correspond to what are usually called round cells. They often appear sheathing the larger blood-vessels of the cortex. The genesis of these foci is obscure, but they are probably due to *Klossiella muris*, as we have not seen them excepting in the presence of this parasite. It is probable that they represent older foci of invasion which had been abandoned by the ripe spores, and the devastated tubules have become the seat of an interstitial process which in its progress may envelope and obliterate neighboring normal tubules as well. Processes associated with acute inflammatory reactions were not encountered. Polynuclear leucocytes as well as exudations were absent.

SYSTEMATIC POSITION OF THE RENAL PARASITE.

The classification of the Coccidia has undergone more or less change in recent years. The last attempt by Schaudinn[1] following Léger is perhaps the best. Of the 3 families there recognized, *Disporo-*

[1] Untersuchungen üb. d. Generationswechsel bei Coccidien. *Zool. Jahrbuch., Abth. f. Morphol.*, 1900, xiii, p. 197.

cystidea, Tetrasporocystidea, and *Polysporocystidea*, the mouse parasite clearly belongs to the third. When we come to the genera our difficulty begins, for these are based on the contents of the individual spore. Sporocysts with 1, 2, 3 and 4 sporozoites are accounted for, but our parasite contains about 30 sporozoites. We have therefore preferred to create a new genus rather than enlarge the scope of any of the existing genera for its reception. We have named the new genus *Klossiella*,[8] of which the type and only known species is *Klossiella muris*. The following is offered as a diagnostic description of the same:

Klossiella muris, gen. et spec. nov. Sporogonic cycle characterized by the development of 12 to 14 spheroidal spores, measuring 16 μ by 13 μ, each of which contains 30 to 34 banana-shaped sporozoites.

Schizogonic cycle unknown, but a phase of unknown significance in the glomerulus.

Sporont with 10 to 20 spherical plastin granules, 1.5 μ in diameter, and smaller granules of the same physical and chemical properties. The latter persist until sporozoite-formation.

Habitat: Renal epithelium (convoluted tubules and glomerulus) of *Mus musculus*.

DESCRIPTION OF PLATES XXI-XXIII.

(The figures on Plate XXI are reproduced from photomicrographs made with the kind permission of Dr. J. H. Wright by Mr. L. S. Brown in the Clinico-pathological Laboratory of the Massachusetts General Hospital. The figures on Plates XXII and XXIII are from camera lucida drawings made with a Leitz 1/12 homogeneous immersion objective. The fixing agent in all cases was Zenker's fluid.)

PLATE XXI.

Fig. 1. Photomicrograph of an area in a section of the cortex of a heavily-infected kidney, showing *Klossiella muris* in the mother- and daughter-sporoblast stages. In the lower right-hand corner is a portion of a glomerulus, showing two parasites in different stages in the epithelium of Bowman's capsule. × 750.

Fig. 2. Photomicrograph of an area of the cortex of the same kidney, showing sporocysts (spores). Some have resisted decolorization and appear

[8] We are indebted to Dr. C. W. Stiles for suggesting to us the generic name *Klossiella* in place of the less accurate Microklossia which we had selected.

as black balls. In others the nuclei of the sporozoites can be made out distinctly. × 800. (Both stained in ferric-alum hæmatoxylin.)

Plate XXII.

Fig. 3. An infected cell from a fresh kidney, teased in salt solution and stained with aceto-methyl green. It contains a sporont within a large vacuole. The spherical refractive bodies in the sporont are plastin granules. × 850.

Fig. 4. A sporont containing both large and small granules. In salt solution; unstained. × 850.

Fig. 5. A double infection. The nucleus has already divided and become distributed towards the periphery. Hæmalum. × 737.

Fig. 6. Mother-sporoblast, showing nuclei near periphery, containing one to four karyosomes and a mass of granular, less intensely-staining chromatin. Hæmalum. × 1435.

Fig. 7. Longitudinal section of a portion of a convoluted tubule, with three infected cells almost occluding its lumen. The two cells to the right show the attachment of the infected cell by a narrow densely-granular peduncle to the basement membrane. The increase in size of the infected cells is clearly seen. Hæmalum and picro-acid fuchsin. × 870.

Figs. 8 and 9. Nuclei of mother-sporoblast stage. × 1700.

Fig. 10. Mother-sporoblast, with daughter-sporoblasts beginning to form. Shrinkage during fixation is probably responsible for the protrusion of one sporoblast beyond the normal contour. Hæmalum. × 1475.

Fig. 11. A stage a little later than the preceding. The daughter-sporoblasts, each with its nucleus at its distal end, appear to bud out from a large central mass. Ferric-alum hæmatoxylin. × 1590.

Fig. 12. The daughter-sporoblasts nearly complete, still attached by a narrow peduncle to central restiform body. Ferric-alum hæmatoxylin. × 1590.

Plate XXIII.

Fig. 13. Transverse section of convoluted tubule, with very large flask-shaped cell, containing *Klossiella* in the daughter-sporoblast stage. The infected cell nearly fills the lumen. It still retains its attachment to basement membrane by a narrow peduncle. Hæmalum; picro-acid fuchsin. × 870.

Fig. 14. Infected cell from fresh kidney, in salt solution. It contains 14 sporoblasts in which the small plastin granules are conspicuous. The cell is more or less flattened by pressure of the cover-glass. × 850.

Fig. 15. Small brood of sporoblasts, showing division and distribution of nuclei preparatory to formation of sporozoites. Hæmalum; picro-acid fuchsin. × 870.

Fig. 16. Brood of spores still within cell-membrane of the completely atrophied cell. Six spores show the sporozoites within the cyst-wall; four are solidly stained and opaque. Ferric-alum hæmatoxylin. × 870.

Fig. 17. Spore from fresh kidney, in salt solution, showing sporozoites still attached to restiform body. × 850.

Fig. 18. More advanced spore, fresh. Aceto-methyl green. Transverse optical section. The nucleus appears in four sporozoites. × 850.

Fig. 19. *a*, *b*, two sporozoites in fresh condition, liberated from spore cyst by crushing; *c*, sporozoite from a spore softened by peptic digestion. × 850.

Fig. 20. Section of glomerulus, showing glomerular parasite *in situ* in visceral layer of Bowman's capsule. × 485.

Fig. 21. Falciform bodies, oldest stage of glomerular parasite observed. Hæmalum, picro-acid fuchsin. × 1590.

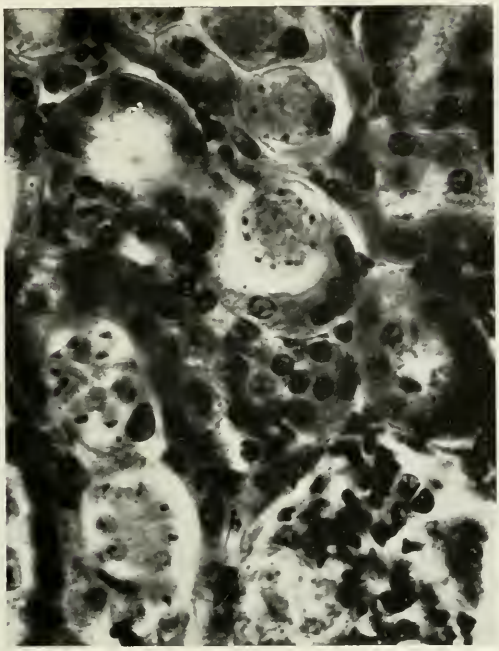


FIG 1.

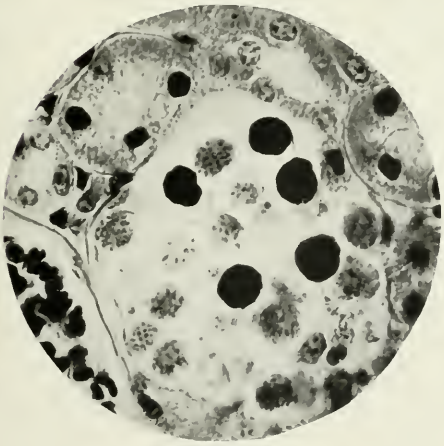


FIG. 2.



3.



5.



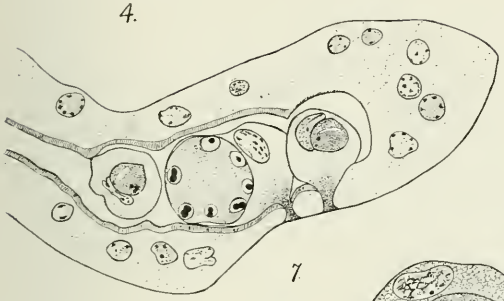
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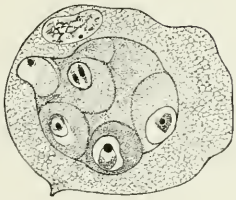
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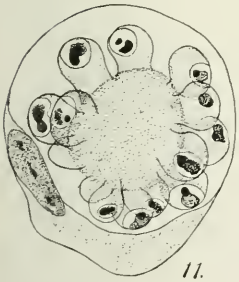
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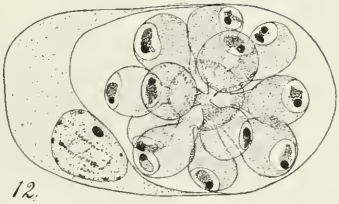
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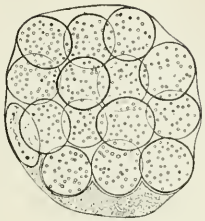


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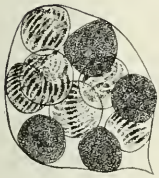
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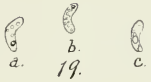
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17.



18.



d. b. c.

19.



H.P.J. del. 20.



21.

A CONTRIBUTION TO THE PHYSIOLOGICAL DIFFERENTIATION OF PNEUMOCOCCUS AND STREPTOCOCCUS, AND TO METHODS OF STAINING CAPSULES.[1,2]

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GENERAL CONSIDERATIONS.

Pneumococci and streptococci which do not differ in morphology from their classic types can usually be differentiated from each other and identified by their morphological characters without difficulty; but it is equally true that certain cultures of these organisms, either

[1] An abstract of this paper was read at the Chicago meeting of The Society of American Bacteriologists, December 31, 1901, and published in *Ctbl. f. Bakt.*, 1902, xxxi, 302; and in *Science*, Mar. 7, 1902, 367.

[2] Received for publication March 18, 1902.

at the time of their isolation or after cultivation on artificial media, approach the type of the other so closely that it may be impossible to identify them by their morphology alone. When such morphological variations occur there are no constant and distinctive cultural or pathogenic characters as yet demonstrated, which can with certainty be depended upon as distinguishing marks between these organisms.[3]

This lack of distinct cultural differences between pneumococci and streptococci has not infrequently led to confusion, but that uncertainty should exist and mistakes be made in identification is not surprising when one considers the characters usually depended upon to distinguish pneumococci from streptococci. Chief among these, as has just been implied, are the morphological features, which are in the case of the pneumococcus a slightly lancet or elongated variation of the more typical coccus form which is characteristic of the streptococci, the arrangement of such cocci in pairs rather than in chains, and the possession of a more or less well-defined capsule. All of these characters are subject to variation or may be absent. Compared with the morphological, the cultural characters are of minor importance, and are variable. They consist in a more watery appearance of the pneumococcus colonies on coagulated blood serum and on agar, and in the usual inability of the freshly isolated pneumococcus to develop readily or at all on gelatin at temperatures below 22° C.

The distinctness of the capsule of the pneumococcus in the body fluids of man and animals, and at times when this organism is artificially cultivated in blood serum, milk or serum agar, has really been depended upon as the chief distinguishing and diagnostic character.

During the past few years, however, from time to time, instances have been reported of distinct capsule formation by organisms which had either been previously identified as streptococcus pyogenes, or

[3] On this subject see Welch, *Bulletin of the Johns Hopkins Hospital*, 1892, December, p. 125 et seq.

at the time of their isolation could not be definitely identified by their discoverers as belonging to either this group or to the pneumococci, but were considered intermediate in their character.

Brief Description of Organisms Reported as Capsulated Streptococci.—Bordet (1) working with an organism previously identified as streptococcus pyogenes described such capsule formation occurring in the peritoneal exudate of infected rabbits.

Schütz's *diplokokkus der Brustseuche der Pferde* (2), Poels and Nolen's streptococcus of contagious pneumonia of cattle (3), and especially the organism described by Bonome (4) as *"streptokokkus der Meningitis cerebro-spinalis epidemica,"* may all be looked upon as organisms differentiated on insecure grounds from either pneumococcus or streptococcus. The first two of these organisms, however, are said to be decolorized by Gram's method, and as suggested by Frosch and Kolle (5) in the case of Schütz's organism, may belong to a group intermediate between Fraenkel's diplococcus and the chicken cholera group.

Tavel and Krumbein (6) describe a streptococcus with a capsule, which was isolated from a small abscess on the finger of a child. Capsules were also present in the artificial cultures, and although ordinarily remaining uncolored, could be stained by Loeffler's flagella stain. This organism was said to be differentiated from Fraenkel's diplococcus and also in general from the streptococci (pyogenes) by a rapid and rich growth on gelatin, agar, and potato. A pellicle was formed on broth. The organisms forming this pellicle possessed capsules, but those in the deeper portions of the broth generally lacked the capsule.

In 1897, Binaghi (7) described a capsulated streptococcus isolated from a guinea-pig dead of a spontaneous peribronchitis and multiple pulmonary abscesses. In the pus were found some diplococci and short chains (4 to 6) surrounded by a capsule, which could be made evident by staining with carbol fuchsin. This organism he proposes to call Streptococcus capsulatus.

Le Roy des Barres and Weinberg (8) in 1899 published an account of a streptococcus with a capsule. This was isolated from a man who had apparently been infected from a horse which had died of an acute intestinal disorder. The patient neglected the infection and died. Diplococci and short chains furnished with a capsule were found in the subcutaneous tissue at the area of infection. The blood, liver and spleen also contained these organisms. The capsule in all the preparations remained uncolored, but the authors say that its existence was not to be doubted.

Ascitic broth inoculated from the peritoneal exudate of a rabbit dying from the infection gave streptococci in extremely long chains and surrounded by capsules. These were not so distinct as in the case of the organisms in the original smear preparations. Nothing notable was observed in the cultural characters. All fluid media (bouillon, milk and ascitic broth) were said to be strongly acid after twenty-four hours. These authors report that Achard and Marmorek have assured them that they have seen capsulated streptococci, and that Marmorek showed them some preparations in which one of his streptococci presented the same characters as that isolated by them.

Although Le Roy des Barres and Weinberg have used the term encapsulated, they believe that it would perhaps be more prudent to call their organism *streptocoque auréolé*, since they were not able to put this capsule definitely in evidence by staining it.

Howard and Perkins (9) have lately described an organism, probably of the foregoing type, which was present in a tubo-ovarian abscess and in the peritoneal exudate, the blood and some of the organs of a woman dying in the Lakeside Hospital, Cleveland, Ohio. The organisms were biscuit-shaped cocci in pairs, usually arranged in chains of four, six, eight or twenty elements, and surrounded by a wide and sharply staining capsule. In the artificial cultures special capsule stains, it was noted, failed to stain any definite area, but numerous small deeply stained granules were to be seen within the halo, especially near its outer border. Capsules in litmus-milk could be sharply stained. Howard and Perkins propose for the group composed of the streptococci of Bonome, Binaghi, and their own organism, the name Streptococcus mucosus.[4]

Reference to the original descriptions of these various capsulated streptococci will show that, with the exception of a rather poorly staining capsule, the majority of these organisms are separated from the typical streptococcus pyogenes or from the pneumococcus by exceedingly slight and unstable morphological and cultural characters. The same is true of the difference observed in their pathogenic action in animals.

There are occasions, then, both within the animal body and in artificial cultivations when it is practically impossible to distinguish definitely between some races of pneumococci and races of strepto-

[4] Through the kindness of Dr. Perkins, I have had an opportunity of studying the organism of Howard and Perkins. It ferments inulin and in most characters shows a closer affinity with pneumococci, than with true streptococci.

cocci. This difficulty is especially heightened when the pneumococcus has become non-virulent, and at the same time no very typical morphology or capsule formation is to be determined and a tendency to chain formation is marked. Cultures of pneumococci in such condition have come under my notice and were not readily to be distinguished morphologically from streptococcus cultures.

Under these circumstances there has been up to the present time no means of determining morphological or cultural differences by which these organisms could be definitely distinguished. The only recourse is the tedious attempt to revivify the pathogenicity by introduction of the culture into the most susceptible animals and to thus bring again into prominence the lancet-shaped diplococcus type, surrounded by the capsules which characterize the pneumococcus in animal fluids.

My work during the past two years with many cultures of pneumococci and streptococci from various sources has shown certain physiological differences in these organisms, which are, so far as I am able to determine, fixed characters, and have thus far proved unfailing means of distinguishing pneumococci from any streptococcus pyogenes met with. It is, of course, understood that the streptococci here referred to are organisms which would ordinarily be classed as streptococcus pyogenes (longus, brevis, conglomeratus), erysipelatos, or scarlatinæ.

In the course of these experiments I have also been led to the development of simple staining methods for capsules, and to the application of special means, cultural and otherwise, of demonstrating the presence of capsules on both pneumococci and streptococci.

It is the object of the present paper to set forth as briefly as may be these differential and capsule experiments.

THE PHYSIOLOGICAL DIFFERENTIATION OF PNEUMOCOCCUS FROM STREPTOCOCCUS.

Preliminary Observations Leading to the Experiments in Differentiation.—My attention was first called to the possibility of a cultural difference between pneumococci and streptococci early in 1900. At

this time I had prepared some serum broth, composed of two parts of broth and one part of an inflammatory exudate from the human pleural cavity. This fluid had originally been rich in cells and was fairly thick, the proteid content being high. Attempts to sterilize at 68° C. had resulted in the solidification or gelatinization of the mixture, and even at 60° C. this was found to take place. The medium was finally sterilized in a fluid condition at 55° C. On March 31, I had occasion to use this medium for the cultivation of a pneumococcus which on March 24 had been isolated from some exudate coming from a case of meningitis in a child following pneumonia. In the serum-broth, after 24 hours' growth at 37° C., the organism brought about a nearly solid coagulum of a yellowish-white color. The culture was examined morphologically by the Welch capsule stain, and was found to be a pure culture of pneumococcus with clearly stained capsules. Subcultures could not be obtained from this tube after 48 hours at 37° C.

The reaction of the uninoculated serum-broth was tested and found to be 0.75% acid, phenolphthalein being the indicator. The coagulated medium containing the pneumococci reacted 2.5% acid. The coagulum dissolved upon the neutralization of the acid.

Shortly subsequent to this, tubes of this same serum-broth were inoculated with two cultures of streptococci. One of the latter was in use in immunizing experiments at the Research Laboratory of the New York Health Department, and the other was isolated by me from the exudate of an empyaema in a child. These, it was noted, did not coagulate the medium even after some days' growth at 37° C.

I was impressed with this difference in coagulative action of the pneumococcus and streptococci in this medium and, realizing that it might indicate more than a temporary difference in the metabolism of these organisms and might thus be of diagnostic value, I made further tests and endeavored to analyze the phenomenon. This, it seemed, might depend upon at least two things—either it might be due to an acid formed by the pneumococcus in the presence of some fermentable substance (probably carbohydrate) not available for the streptococci, or it might be due to an acid produced by the pneumococcus quite independent of a carbohydrate in its nutrient surroundings. That acid was formed and that the precipitate was due in large part to this was indicated by the titration and by the resolving of the coagulum upon the addition of an alkali.

What the fermentable substance was, if there really was one present, which is not unlikely, was not determined.

The normal amount of glucose in fresh beef serum, when the serum is mixed with broth in the proportion of 1 to 2 and the glucose fermented by organisms known to act upon it, such as B. coli communis and B. typhosus, does not lead to such a coagulation, neither did these organisms give a coagulum when grown in the inflammatory serum broth. It seemed, therefore, that this substance, if it were a carbohydrate, must be one of the less readily fermented, such, for instance, as glycogen; or it might be that some glucoproteid or possibly nucleoproteid, which could be broken up by the pneumococcus, was present in excess of the usual amount. This latter supposition is not unreasonable, as the cell content of the serum was large.

It did not seem probable that glycogen, even if originally present, could long remain in such a serum unchanged by the diastasic and maltasic ferments, which are normally present in blood and such exudates. Experiments were, however, carried on with various carbohydrates, with the hope of thus throwing some light on the problem, and they have led to such interesting and, it is hoped, valuable results, that they form an important part of the present communication.

No attempt has as yet been made to investigate the glucoproteids and nucleoproteids.

Culture Experiments with Serum-Broth Media, Sterilized at 65°-70° C.—In the first attempt to devise a differential culture medium, fresh beef-serum was diluted with sugar-free broth, reacting 1% acid[5] to phenolphthalein, in the proportion of two parts of broth to one part of serum. This was then divided into separate portions, one of which was left plain, and to the others were added respectively dextrose 1%, lactose 1%, saccharose 1%, dextrin 1% and starch ⅔%.

These media were sterilized at 65°-68° C. for one hour on six consecutive days. They were inoculated with two cultures of pneumococci and four of streptococci.

The dextrose, lactose, saccharose and dextrin media were found to be fermented by the streptococci as well as by the pneumococci, with the formation of a solid, yellowish-white coagulum, due to the resulting acid.

[5] This broth was neutral to litmus.

The starch medium yielded the most satisfactory differential results. This medium was rapidly fermented by the pneumococci, but was noted only after 13 days' growth at 37° C. as becoming gelatinous from the action of the streptococcus cultures. The plain serum was not coagulated by either organism. Anaërobic cultivations were made. The results were practically the same as given by aërobic growth with the exception of the coagulation of the plain serum-broth by the pneumococcus.

The results indicated by the starch medium were promising. They showed that these starch preparations could be readily fermented by pneumococci with rapid acid formation, and that the streptococci, on the other hand, although they developed in the medium, could not avail themselves of the starch, at least with ease, for it was generally a matter of many days before even a gelatinization of the medium resulted.

As there was, as determined by the iodine test and by the use of Fehling's solution, a marked conversion of the starch during the preparation of the media, and by this the introduction of complicating factors into the tests, changes were made in the mode of preparing the medium.

Culture Experiments with Serum-Water Media, Sterilized at 65°-70° C.—Distilled water was substituted for the broth in these experiments so as to exclude any hydrolization in the presence of acids and salts, such as are usually present in broth, during the preparation of the starch.

Starch water was prepared by adding 4 grams of powdered starch to 400 cc. of water, boiling for one-half hour, and allowing to stand over night. In the morning a clear fluid could be obtained by pipetting off the water from which the starch particles had settled to the bottom of the flask. This was then added to serum in the proportions of serum 1 part, starch water 2 parts, and sterilized at 68° C. for 1 hour on 6 consecutive days. A medium containing glycogen 1% was also made.

A test of these media showed that in both of them the pneumococcus not only grew readily but induced a coagulum in the glycogen as well as in the starch medium, and this often within 24 hours, at 37° C. The streptococcus cultures grew well, but, as in the first experiment, did not coagulate the starch medium readily, nor bring about coagulation of the glycogen serum, except after many days, and in some cases apparently were not able to do this at all.

Having proved by this preliminary experiment that growth of pneumococci and streptococci would take place in serum diluted with distilled water, it was possible thereafter to avoid the complicating factors incident to the use of broth.

Besides the starch and glycogen media already mentioned, tests were made also with aqueous serum media plus dextrose, lactose, galactose, maltose, saccharose. All of these mono- di- and polysaccharids were readily and rapidly used by the pneumococci, and gave rise in all cases to acid sufficient to cause the coagulation of the albuminous material in the serum. The streptococcus cultures as a rule readily fermented dextrose, galactose, and the maltose (commercial specimen), less readily lactose and saccharose; but starch and glycogen, as noted above, if changed at all, were usually only sufficiently affected by the streptococci to give rise to coagulation after many days and after some evaporation from remaining at 37° C.

These results indicated a marked difference in degree, but not necessarily in kind, between the fermentative power of the pneumococcus and streptococcus cultures as a class. No perfectly distinct differentiation, however, had so far been obtained between the pneumococcus and all of the streptococcus cultures tested.

Culture Experiments with Alkaline Serum-Water Media, Sterilized at 100° C.—It was realized that diastasic action going on during the preparation of the media might affect the result of these experiments. Changes might thus be brought about either by the diastase and maltase (glucase) and glycolylic ferments of the

blood or by the action of contaminating bacteria or their accompanying ferments during the long process of low temperature sterilization at 65°-70° C.

The diastasic action of beef serum, as was demonstrated in some experiments, in such mixtures as those used by me, was exceedingly rapid and well marked, as was also the action of maltase. Starch serum mixtures rapidly failed to give the blue reaction with iodine, and after standing for a few hours or even less at the room temperature failed to give even the brown reaction indicating the presence of erythrodextrin. Those heated to 65°-70° C. failed in these reactions even sooner. This change must be equally true in the case of glycogen, and indicated that our results were not necessarily due to the action of our organism on pure starch or glycogen, but probably on some product of the fermentation of these substances by the enzymes of the blood or by bacteria present during their preparation. Aside from these complications, low temperature sterilizations are at best tedious and unsatisfactory. An attempt was therefore made to eliminate these factors of uncertainty in the preparation of the media.

The first experiment was made by adding small amounts of alkali to the serum-water media, made as above by adding 1 part of beef-serum to 2 parts of distilled water, with the object of converting the proteids into alkali albuminates which could be boiled without coagulating.

By the addition of 1 cc. *n/1* NaOH to the serum water, a perfectly clear fluid resulted which could be sterilized at 100° C., and remain uncoagulated and clear. One-half per cent *n/1* NaOH also gave a non-coagulable medium, but was not quite as clear after boiling as the 1%. Such media are, however, open to the objection that the sugars or starches may be changed when boiled in the presence of an alkali. Gradual reductions of the alkali content were therefore practised and it was eventually found that no added alkali was needed to prevent coagulation in mixtures in the proportion of one part of ox-serum to two parts of distilled water. Such a medium,

although becoming opalescent at boiling temperature, remains perfectly fluid, and is available as a nutrient base for such sugar tests.

Before these last results were obtained, tests were made with the alkaline media plus some of the sugars, and also with the plain alkaline medium. The sugar tests showed nothing especially worth noting except that a longer interval elapsed before the coagulation of the albumins took place, this being due to the additional alkali which had to be neutralized.

The chief point of interest and value developed in connection with the use of a medium without sugar but which had been rendered slightly alkaline by the addition of 0.2% of *n/1* NaOH. In this medium the pneumococcus cultures invariably gave rise to an opalescence, a gradual whitening and final gelatinization and coagulation. The amount of alkali was afterwards reduced to 0.1% *n/1* NaOH, and this medium was found to give rise when grown in the incubator at 37° C. to the same opalescence and whitening after about 48 hours, with a firm coagulation after some days, depending upon the culture tested. As may be seen in the appended Table I, no streptococcus cultures bring about visible changes in this medium.

We thus have in this alkaline medium, made of one part of ox-serum and two parts of distilled water plus 0.1% n/1 NaOH and sterilized at 100° C., a means of differentiating the pneumococcus from various cultures of streptococci, and so far as my experience goes from all. The behavior of the pneumococcus in this medium seems to indicate a fundamental difference in its metabolism from that of the streptococci. Upon what metabolic process and nutrient ingredients this formation of acid depends has not as yet been determined.

TABLE I.

TEST OF PNEUMOCOCCI AND STREPTOCOCCI IN THE ALKALINE SERUM WATER MEDIUM. 0.1% *n/1* NaOH.

DAYS.	PNEUMOCOCCUS CULTURES.					STREPTOCOCCUS CULTURES.
	V	VI	VII	VIII	IX	
1	..	..	..	..	..	Twenty-eight cultures were tested; none of these brought about any visible change in the medium.
2	Becoming opalescent.	Becoming opalescent.	..	Becoming opalescent.	..	
3	White.	White.	Becoming opalescent.	White.	..	
4	Gelatinous.	Gelatinous.	White.	Gelatinous.	Becoming opalescent.	
5	Semisolid.	Semisolid.	"	Semisolid.	White	
6	"	"	"	"	"	
7	"	"	"	"	"	
8	+	+	Gelatinous.	+	Gelatinous.	
9	..	..	+	..	Semisolid.	
10	..	..	..	..	+	
20	..	..	..	..	..	

+ Indicates a solid coagulum.

Culture Experiments with Serum-Water Media, Sterilized at 100° C.—After it had been observed that a mixture of one part of beef-serum and two parts of distilled water could be boiled and thus sterilized without precipitating the albuminous materials present, media were prepared in this manner, containing 1% of various carbohydrates—thus, dextrose, galactose, maltose, lactose, saccharose, dextrin, starch, glycogen, and inulin. These carbohydrates, with the exception of the maltose, which was a fair commercial sample, were of high purity, the galactose, lactose and glycogen[6] having been especially prepared and tested for this work. Such precautions, it need hardly be remarked, are absolutely essential to the success of these experiments. Samples of glycogen made by manufacturers, and bought in the open market, gave results totally at variance with those obtained when the specially prepared glycogen was tested. More readily fermentable carbohydrates were always found to be present with the glycogen in manufacturers' samples.

This series of experiments with the carbohydrates showed that the monosaccharids — dextrose, levulose, galactose — were fermented readily by practically all streptococci as well as by the pneumococci. This is true also of the disaccharids—maltose, lactose, saccharose—as regards the streptococci as a class, but the development of the coagulum may be much slower than in the case of the monosaccharids, and with certain cultures of streptococci it may be will not occur at all.[7] In the case of the polysaccharids—dextrin, starch and glycogen—coagulation usually takes place only after many days in streptococcus cultures, and in some cultures apparently does not occur. The non-coagulation is more frequent with glycogen than with starch. Inulin, however, is not fermented by any of the streptococci, although it is readily used by the pneumococci in such serum media. Growing in such an inulin medium, the pneumococcus rapidly gives rise to acid, which leads to the formation of a solid white coagulum which is

[6] These samples of glycogen, lactose and galactose were especially prepared for me by Dr. A. N. Richards, to whom my sincere thanks are due.

[7] This subject will be discussed more fully in a later paper on the fermentative power of the streptococcus group.

usually complete within 48 hours. By the use of this medium a rapid and constant differentiation can be obtained between pneumococci and the streptococcus pyogenes group.

The differences in behavior of the streptococci in inulin from their behavior in starch and glycogen—the constancy of the non-coagulation—may in part be accounted for by the character of the inulin itself. Inulin is obtained chemically pure and is therefore free from contamination with more readily fermentable carbohydrates. Furthermore, it apparently is not hydrolized during boiling in such a serum medium, nor is it affected by the diastasic ferment present in the blood serum. Glycogen, on the other hand, is only obtained in a comparatively pure state with difficulty, is probably hydrolized to some extent by boiling after it is added to the medium, and is readily acted upon by the diastase of the blood, and subsequently by the maltase, if the preparation be not rapidly carried on or the serum-water heated previous to its addition. These facts may account for some discrepant results, although some are most likely due to differences in action of the streptococci, and may indicate fundamental differences in their physiology.

Preparation of inulin serum-water medium.—The inulin medium is satisfactorily prepared as follows: To one part of fresh, clear beef-serum is added two parts of distilled water, and to this mixture is added 1% of pure inulin. The inulin goes into solution slowly in the cold, and the mixture may be warmed to 55°-60° C. to hasten this solution.[8] As soon as the inulin is dissolved, the medium should be tubed, and sterilized immediately at 100° C. for ten minutes.[9] This sterilization is repeated on the two following days. The medium prepared in this way usually becomes only slightly turbid, and no unfavorable changes occur in it.

While this is a favorable method of preparing the inulin medium and no hydrolization or change of the inulin is effected by the action

[8] The inulin may be dissolved in the distilled water before it is added to the serum.

[9] Very resistant spores are at times present in inulin. The usual three heatings are then not sufficient, and the medium should be sterilized in the autoclave, at 10 to 15 pounds for 15 minutes.

of the enzymes of the serum, it may also be prepared by first heating the serum-water mixture to 100° C. for some minutes and then adding the inulin. This method should always be followed in preparing other carbohydrate serum-water media. Litmus solution (Merck's highly purified litmus, 5% solution in distilled water) may be added to this media in the proportion of 1%, and changes in reaction thus detected by the color change.

Since sera, even from the same species of animal, differ at times in their initial degree of alkalinity and salt content, specimens are met with, though rarely, which are of so low an alkalinity or have so high a salt content that media made from them are extremely white and opaque, and near the point of heat coagulation. Such media should not be used.

The results of tests of various pneumococcus and streptococcus cultures in 1% inulin serum are shown in Table II. It will be seen that the pneumococcus cultures tested usually coagulated the serum within 48 hours, while the streptococcus cultures, 28 of which were tested, were without effect, although all of them grew at least feebly in the medium.

Summary of Culture Experiments.—It is plain from the foregoing experiments that the pneumococcus has a remarkable ability to utilize carbohydrates in its metabolic processes—all mono-, di- and polysaccharids that were tested being rapidly fermented by it as demonstrated by the production of acid. Further than this, the pneumococcus can produce sufficient acid to give rise to a coagulum in a serum medium which is sugar free, or which at least does not contain enough fermentable saccharine matter for the production of appreciable acid by such well-known fermenting organisms as the colon bacillus and the typhoid bacillus, which readily give rise to acid in the presence of glucose.

The streptococci, on the other hand, have apparently more limited and certainly less active fermentative powers. While they usually ferment the monosaccharids and in most instances the disaccharids

TABLE II.

TEST OF PNEUMOCOCCI AND STREPTOCOCCI IN THE INULIN SERUM-WATER MEDIUM.

DAYS.	PNEUMOCOCCUS CULTURES.									STREPTOCOCCUS CULTURES.
	V	VI	VII	VIII	IX	X	XI	XII	XIII	
1	+	+	+	..	..	..	+	+	..	Twenty-eight cultures were tested; none of these brought about any visible change in the medium.
2	..	..	..	+	+	..	..	..	+	
3	..	..	..	..	.	Gelatinous.	..	..	..	
4	..	..	..	..	..	"	..	..	..	
5	..	..	..	..	..	"	..	..	..	
6	..	..	..	..	..	"	..	..	..	
7	..	..	..	..	..	"	..	..	..	
8	..	..	..	..	..	+	..	..	..	
9	..	..	..	..	..	..	..	..	..	
10	..	..	..	..	..	..	..	..	..	
20	..	..	..	..	..	..	..	..	..	

+ Indicates a solid coagulum.

rapidly and with ease, the polysaccharids they either do not ferment at all or very slowly. Glycogen and starch are fermented only by certain species or races of the streptococci; inulin is fermented by none so far tested. Furthermore, an appreciable amount of acid is not produced in sugar-free serum media by any streptococcus pyogenes so far as our experience goes.

We may assume, therefore—although one hesitates to dogmatize even after extended experiments on a group of organisms whose physiology is so little understood—that there are distinct differences in respect to the physiological processes of these two forms, or to speak more correctly, of the pneumococcus on one side and the streptococcus group on the other.

The relationship of these organisms may be compared to that which exists between typhoid bacilli and the bacilli of the Gärtner-colon group. The pneumococci resemble the typhoid bacilli in the distinctness and apparent permanence of their cultural characters, while the streptococci seem to form a group which resembles the Gärtner-colon group in presenting grades of fermentative activity, and especially as this activity is displayed by the streptococci in such aqueous serum media as those used in these experiments. In making this comparison it is recognized that the pneumococcus is to be known by positive cultural characters as shown by its ability to ferment the various mono-, di- and polysaccharids, while the typhoid bacilli are chiefly distinguished in comparison with the members of the Gärtner-colon group by the absence of certain physiological activities, such as gas and indol production and the coagulation of milk. Still by this comparison it is simply desired to emphasize the co-extensiveness of the characters, as shown by these experiments, of the organisms commonly recognized as pneumococci, as compared with the variety or variability of the characters displayed by organisms which would ordinarily be classed as streptococci, and probably as streptococcus pyogenes.

EXPERIMENTS ON THE DEMONSTRATION AND STAINING OF CAPSULES ON PNEUMOCOCCI AND STREPTOCOCCI.

Welch's stain for capsules was the stain constantly used in the earlier of these experiments, and as a rule gave excellent results. Certain conditions, however, at times arose in some of the cultures which interfered with the success of this stain, and I was thus led to seek other methods of staining and of demonstrating the capsules on pneumococci.

New Methods Devised for Staining Capsules.—After many experiments it was found that a very satisfactory and rapid staining of the pneumococcus capsules could be effected by the following method:

Potassium carbonate method.—This method consists in using as a dye a half-saturated aqueous solution of gentian violet.[10] This is applied for a few seconds to the cover-glass preparation, which has previously been allowed to dry in the air, and has been fixed by heat in the usual manner. No water must be used in diluting and spreading the organisms on the cover-glass. If the organisms come from a solid medium or fluid medium other than fluid sera, some other diluting and spreading fluid such as will be described later must be used. The dye is washed off with a one-fourth per cent (0.25%) aqueous solution of potassium carbonate and the specimen is studied in this fluid. The cover-glass may be sealed on the slide by rimming with vaseline, and evaporation thus prevented. This stain gives remarkable results when used on pneumococci either from fluid or hardened serum media or from the body fluids. (See Figs. 1, 3, 4.) The capsules are large, prominent, and are either stained throughout, or their periphery appears as a dark line or layer, the part next to the deeply stained diplococci being less intensely stained than the periphery. This appearance may be due in part to a precipitation of the dye on the exterior of the capsules by the potassium carbonate.

[10] Gentian violet in substance is added in excess to distilled water and allowed to dissolve to its full extent. The solution is then filtered, and diluted to twice its volume.

Very fair capsule stains may also be obtained by simply staining for about thirty seconds with the ordinary aqueous gentian violet solution (5 cc. sat. alc. sol. to 95 cc. of distilled water) and then washing with a 1% potassium carbonate solution and studying the specimen in the fluid.

Preparations stained by the potassium carbonate method may at times give satisfactory results when dried and mounted in balsam (preferably gum Damar), but this is not the rule.

The following method, however, is eminently satisfactory when it is desired to mount specimens of pneumococci with capsules in balsam.

Copper sulphate method.—In this method a 5% or 10% aqueous solution of gentian violet or fuchsin (5 cc. sat. alc. sol. to 95 cc. distilled water) is placed on the preparation, which has been prepared as previously indicated. The dye is allowed to steam for a few seconds, by gradually passing the cover-glass downwards through the flame several times. After this the staining fluid is washed off with a 20% solution of copper sulphate. This solution may vary from 10% to saturation, but the medium strength, 20%, is generally satisfactory. After washing with copper sulphate, the preparation is dried between filter papers and when thoroughly dry mounted in balsam. The capsules remain of normal size, stained, and distinct. (Figure 2.)

Observations and Experiments on Media and Conditions Favorable to Capsule Development and Staining.—The most favorable conditions known for the development of the pneumococcus capsule are found in body fluids of man and animals suffering from an infection with this organism. For instance, capsules may be demonstrated with ease by the usual methods in the blood, serum and inflammatory exudates of the infected rabbit which is, among test animals, one of the most favorable for these experiments. Capsules may be equally well marked in the fresh sputum of pneumonia patients, especially in the early stages of the disease, and in the exudates accompanying such pneumococcus infections as meningitis, otitis media, and empyaema. In sputum and the exudates of these

various localized infections the organisms are, however, frequently degenerated or under chemical conditions unfavorable for capsule staining, and satisfactory results are then not easily to be obtained. The same is true of the scrapings from lungs of patients dead of pneumonia, often even in the stage of red hepatization. Under these conditions a longer exposure to the staining reagent is necessary, before the organisms and capsules are brought into prominence, and even then the results in nowise compare to those obtained with organisms in fresh sputum or the body fluids of such an animal as the rabbit.

It was shown by Ortman (10), as early as 1888, that outside of the animal or human body pneumococci regularly developed capsules when cultivated in blood serum. Welch [11] in 1892 and Paulsen (11) in 1893 called attention to the development of capsules on these organisms in milk, and Schmidt (12) showed that sputum media favored the formation or preservation of pneumococcus capsules. Schabad (13) made a similar observation in 1896 in regard to blood agar. Frosch and Kolle (14) refer to the demonstration of capsules on pneumococci cultivated in Guarnieri's medium, and rather indefinite statements in regard to capsules on pneumococci coming from broth and other cultivations may be found in various articles on the pneumococci.

In my own work, most of these experiments have been repeated and the results confirmed. In the serum media of various composition used during the experiments on the physiological differentiation of pneumococci and streptococci, the pneumococci not only grew readily but developed distinct and well-marked capsules. This capsule formation seems to be independent of the length of time the organism has been under artificial cultivation. One of the most favorable media of all for the development of capsules was that made of 1% starch-bouillon and serum—serum 1 part, bouillon plus 1% starch 2 parts—and sterilized at 65°-70° C. In this medium, according to my experience, capsules are developed with great regu-

[11] loc. cit.

larity and may be stained without difficulty. The same is true of pneumococci grown on Loeffler's coagulated blood serum, or on coagulated serum without glucose; and these organisms may be prepared for staining by simply spreading them on the cover-glass in some of the condensation water from the serum tube. This use of condensation water may also be successful in the case of organisms growing on plain or glycerine agar, but the most successful method to preserve or perhaps to accentuate the capsule on pneumococci or streptococci coming from artificial media, other than fluid serum media, is to use a drop of serum as the diluting and spreading fluid for the cover-glass preparations. Some of the carbohydrate serum mixtures, such as the starch or glycogen media and the alkaline media, often serve this purpose better than unmodified or fresh serum.

My first experiments in this direction were with the serum starch mixtures used for cultural purposes. It occurred to me that such a fluid in which the pneumococcus capsules were always present and demonstrable by staining might serve to preserve or even develop them on organisms when these were transferred to a drop of the fluid from various artificial media, such as broth, agar, etc. This is successful with pneumococcus nearly without exception, and the presence of a capsule bears little relation to the time the organism has been cultivated artificially, or the medium from which it comes. It is well, however, to have fresh cultures, say usually not over 24 hours old.

It is to be noted that such a fixing medium does not bring the capsule into prominence by simply serving as a deeply stained background and leaving unstained around the organisms the so-called "retraction zone." By the potassium carbonate method the diluting medium often remains practically unstained, while the capsule stands out plainly, either stained definitely throughout, or with a distinct peripheral line or layer which shows to perfection when the organism is free from surrounding detritus. In the copper sulphate method the capsules stain uniformly while the field may or may not be free from detritus.

In smears from the serum of infected animals, *i. e.*, rabbits with pneumococcus, the potassium carbonate method leaves everything practically unstained but the organisms and their capsules. Of course, if blood cells are present they take the stain to some extent.

With the copper sulphate method the serum is stained, but is contracted into threads and masses, and does not give a uniform background. (Figure 2.)

The following experiment made with a bacillus isolated in company with a streptococcus from a case of endocarditis, but probably having no etiological significance, shows the advantage in staining capsules of preparations made with serum or serum mixtures. A smear was made directly on a cover-slip from an agar culture, no diluting fluid being used. By the potassium carbonate method a small capsule was demonstrated. When, however, it was mixed on the cover-glass with some serum (glycogen serum) and stained by the same method, very large capsules were found. In some instances the bacilli could be seen dislodged from their central position in the capsules and sticking half way out of them; in other instances the bacilli were seen entirely free and lying naked alongside of the empty capsules. The photograph of this specimen was made from a preparation stained with aqueous gentian violet, washed with 0.25% sol. of potassic carbonate, dried and mounted in balsam. (Figure 12.)

Demonstration and Staining of Capsules on Streptococci.—In determining the growth of the streptococci in the various experimental media, the cover-glass preparations were often stained by the same method (K_2CO_3) as that used to demonstrate the capsule of the pneumococcus. In this way the fact was brought to light that the streptococci of the cultures tested were possessed of capsules, which stained or were made visible by these methods. This was especially true of cultures in sugar serum media, and in the more alkaline serum media. In some cultures the capsules were quite as sharp and as well-defined as those of the pneumococcus; in others

they were less well marked, and in some appeared as if in a semi-fluid state and on the point of dissolving. In these last, when several organisms were massed together, their capsules seemed to coalesce. This, however, may occur with pneumococcus capsules, though it is by no means so frequent.

Source of the Streptococcus Cultures Used in these Experiments.— The source of the various streptococci which have been used in the experiments will be of interest in this connection, since, as was noted in the earlier part of this paper, the presence of capsules on certain streptococci has been looked upon as a character sufficient to place them in separate species.

The cultures enumerated are those which have been used in all of the cultural and capsule tests.

No. 1. Isolated from milk, May, 1898. Has been cultivated on artificial media for many months.

No. 2. Isolated from human throat (diphtheria suspected) November 18, 1898. Has been long on artificial media.

No. 3. Isolated from an abscess of a horse following subcutaneous injection of diphtheria toxin (filtered), May 17, 1900. Some months at least on artificial media.

No. 4. Isolated from another antitoxin horse. Nasal discharge due to disease of nasal sinus, probably necrosis of bone. November, 1901.[12]

No. 5. Isolated from a case of suppurative pylephlebitis in man. This organism accompanied a bacillus which was a strict anaërobe.[13]

No. 7. From a case of appendicitis.

No. 8. Culture marked "M" from the Research Laboratory, Department of Health, N. Y. Isolated from a case of erysipelas.

No. 9. Same culture as "8," but grown for many months as a separate culture under slightly different cultural conditions.

No. 10. Said to have come from a case of scarlet fever. Research Laboratory, Department of Health, N. Y. Marked "B."

No. 11. Present in pure culture in the urine of a man suffering from a marked cystitis. Patient gave history of chronic pyuria. Capsules on streptococci in the original urine smears.

[12] I am indebted to Dr. Theobald Smith for these four cultures. They were labelled I, IV, VII, X in the order enumerated in the text.

[13] See Norris, *Journal of Medical Research*, 1901, I, 97. I am also indebted to Dr. Norris for culture 7.

No. 12. Rapid ascending cellulitis of leg. Tissues after 24 hours appeared gangrenous. Patient showed marked signs of sepsis, but eventually recovered.

No. 13. Isolated from the heart blood of a patient showing lesions of endocarditis.

No. 14. Isolated from human uterus. Puerperal sepsis.[14]

These cultures from such widely different sources present the usual characters ascribed to streptococcus pyogenes, and so far as I have studied them, are not to be differentiated from one another by the ordinary cultural tests. Morphologically they do not present more than the ordinary slight differences which may be probably attributed to slight variations in physical and chemical environment, rather than to constant inherent differences in the organisms themselves.

All of them coagulate milk after a varying number of days, and grow in gelatin at the room temperature. Nos. 8, 9 and 10 developed tardily in gelatin, did not extend along the puncture to the surface of the medium, but grew along the lower half of the line of inoculation. All of the other cultures grew readily in gelatin, some of them spreading slightly away from the point of inoculation on the surface of the medium.

The broth cultures were not especially noteworthy, except in the different behavior of the same organism when tested in broth containing sugars, and in sugar-free broth. Either flocculent growth or uniform clouding may occur according to the character of the broth in which cultivations are made. Uniformly clouding non-sugar broth, they may present flocculi in one or more of the sugar broths, or vice versa, according to the culture tested. These characters seem to be independent of the acid produced in the medium. I note, for example, organism "8" *glucose broth:* uniform clouding, dense; *plain broth:* flocculi, medium amount of growth. Organism "12," *plain broth:* flocculi small, practically uniform clouding; *glucose broth:* flocculi large, plentiful sticky growth, organisms in very long chains.

All of the streptococci from the above described sources have been found to possess capsules which become apparent under various conditions when stained by the methods mentioned. Streptococcus No. 11 had a well-marked and easily stained capsule in the original urine smear preparations, and also in some of the artificial cultiva-

[14] See Wadsworth, *American Journal of Obstetrics*, 1901, XLIII, No. 4.

tions, especially that on glycerine agar, and on serum agar. The same is true of streptococcus No. 7.

The photographs of most of the preparations were taken after the organisms had been cultivated for many generations on artificial media. As a rule, the best examples were given by cultures on ascitic serum agar. My best results with streptococci were obtained with organisms coming from serum (ascitic) agar, and diluted on the cover-glass in a drop of glycogen serum mixture which had been sterilized at 68° C., and had undergone evaporation to about one-half of its original volume.[15] It happened that the chemical and physical condition of this mixture was exceedingly favorable to capsule preservation. Most of the photographs of streptococci were taken from specimens prepared in this way, dried in the air, fixed in the flame, and stained by the method in which a half-saturated aqueous solution of gentian violet and 0.25% potassium carbonate solution are used. Fine clear specimens may often be obtained in this way, when not a trace of capsule can be demonstrated on the organism in smears direct from fluid or other artificial media.

These experiments, in which it has been possible to demonstrate capsules in all of the cultures of streptococci tested and this often after long cultivation on artificial media, suggest that to place streptococci which have been found by the usual methods possessed of capsules, in a different specific group from the ordinary streptococci pyogenes is not warranted. And especially is this true if this character is the sole or major distinguishing feature.

In the present or immediately preceding history of such cultures, conditions favoring the development of capsules may have existed and thus brought the capsule formation to a maximum. Such conditions may have been found in the animal or human host, or as in the case examined by me, in such a medium as an albuminous purulent urine. Organisms coming from such sources, and cultivated on artificial media might well display this character for some time sub-

[15] No attempt has as yet been made to reproduce this medium.

sequently more highly developed than it is among their fellows which had not had the same previous history.

I have noticed that pneumococci, which it is well known have particularly well-marked capsules in rabbit blood or serum, have this character, in some instances at least, to a less degree in the serum of guinea-pigs which have succumbed to the pneumococcus infection.

It seems more advisable, therefore, in the present state of our knowledge, to look upon capsule formation as general among streptococci, and of no absolute diagnostic or differential significance. It is also fair to conclude that unless there are cultural differences accompanying this marked development of the capsule on organisms suspected of being streptococci, that such organisms should not be placed in a separate specific group.

GENERAL SUMMARY AND CONCLUSIONS.

By morphological examination and with current cultural methods a clear differentiation cannot always be made between pneumococci and streptococci. The chief differential character usually depended upon is the capsule of the pneumococcus. Well-marked capsules, however, may occur on organisms which have with reason been classified as streptococci. On the other hand, capsules may not be demonstrable on pneumococci by the usual methods, especially when growing on artificial culture media.

The usual cultural characters and reactions are at best not diagnostic, and are subject to variations which may render them useless as evidence of specific difference.

The experiments recorded in this paper, however, afford some evidence that there are well-marked differences between the metabolic activities of pneumococci and streptococci, which may prove useful in the differentiation of these organisms. These differences in metabolism become apparent when the pneumococci and streptococci are cultivated in an alkaline serum medium, or in a serum medium to which the carbohydrate, inulin, has been added.

Pneumococci slowly produce acid in the alkaline serum.

In the inulin media they ferment the inulin and thus rapidly give rise to acid. Streptococci do not form appreciable acid in either of these media, nor do they ferment the inulin.

The differences between the metabolism of pneumococci and streptococci are indicated by visible changes in the media. Thus the alkaline serum and the inulin serum are coagulated by the acid formed during the growth of the pneumococci. This coagulation is rapid in the inulin serum medium, slower in the alkaline serum medium. The streptococci, on the other hand, do not bring about a coagulation of these media.

We have, therefore, in either of these two media, the alkaline or the inulin, so far as our experience goes, a definite means of differentiating pneumococci from streptococci.

Starch and glycogen media, prepared in the same manner as the inulin medium, are coagulated by pneumococci and by some at least of the streptococci. With the streptococci the coagulation, if it occurs at all, is usually long delayed. It may be that some or all of the streptococci do not ferment pure starch and glycogen.

Lactose, saccharose and maltose are fermented by pneumococci with the production of acid, thus giving rise to acid coagula in media containing serum. Certain members, though probably not all, of the Streptococcus pyogenes group ferment these disaccharids—lactose, saccharose, maltose—hence such sugars are not available in media used to differentiate pneumococci from streptococci as a group.

Monosaccharids—dextrose, galactose, possibly all monosaccharids—are fermented by pneumococcus and the various members (probably all) of the streptococcus group. Serum media containing these sugars are rapidly coagulated by the resulting acid.

In serum media, especially starch-bouillon-serum, sterilized at 68° C., pneumococci usually develop well-marked capsules. In some of the serum media, streptococcus cultures may at times have demonstrable capsules.

All streptococci examined, which by the usual methods would be classified as streptococcus pyogenes, have been found to possess cap-

sules. These were demonstrated by special methods and stains devised during this work.

These methods and stains are also especially applicable to the demonstration and staining of pneumococcus capsules.

In the light of the demonstration of capsules on streptococci, the usual morphological basis of differentiation of streptococci from pneumococci appears insecure.

This is also true of the separation of the organisms described as capsulated streptococci into species distinct from streptococcus pyogenes, or from true pneumococci.

This separation does not seem warrantable unless other and especially well-marked cultural differences are demonstrated, which distinguish such capsulated streptococci from pneumococci or from streptococcus pyogenes. Well-marked examples of organisms which would probably be described as capsulated streptococci have been examined during these experiments. They have been found to correspond to streptococcus pyogenes when cultivated in the media described in this paper.

It is a pleasure to acknowledge my indebtedness to Dr. Edward Leaming for the photographs reproduced in the plates.

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DESCRIPTION OF PHOTOGRAPHS.

Plate XXIV.

Fig. 1. Pneumococcus I from Loeffler's blood serum. Spread in condensation water. Stained by potassium carbonate method. 2000 ×.

Fig. 2. Pneumococcus I from heart blood of rabbit. Copper sulphate method. Mounted in balsam. 2000 ×.

Plate XXV.

Fig. 3. Pneumococcus II from serum agar. Spread on cover-glass in serum. Pot. carbonate method. Mounted in K_2CO_3 sol. 1000 ×.

Fig. 4. Pneumococcus I from sugar free broth. Spread on cover-glass in serum. Potassium carbonate method. Mounted in K_2CO_3 sol. 1000 ×.

Fig. 5. Streptococcus I from serum agar. Spread in serum. Potassium carbonate method. Mounted in K_2CO_3 sol. 1000 ×.

Fig. 6. Streptococcus II from serum agar. Spread in serum. Potassium carbonate method. Mounted in K_2CO_3 sol. 1000 ×.

Fig. 7. Streptococcus IV from serum agar. Spread in serum. Potassium carbonate method. Mounted in K_2CO_3 sol. 1000 ×.

Fig. 8. Streptococcus VII from glycerine agar. Spread in serum. Potassium carbonate method. Mounted in K_2CO_3 sol. 1000 ×.

Plate XXVI.

Fig. 9. Streptococcus IX from serum agar. Spread in serum. Potassium carbonate method. Mounted in K_2CO_3 sol. 1000 ×.

Fig. 10. Streptococcus XI from serum agar. Spread in serum. Potassium carbonate method. Mounted in K_2CO_3 sol. 1000 ×.

Fig. 11. Streptococcus XIV from serum agar. Spread in serum. Potassium carbonate method. Mounted in K_2CO_3 sol. 1000 ×.

Fig. 12. Capsulated bacillus from agar. Spread in serum. Potassium carbonate method. Mounted in balsam. 1000 ×.

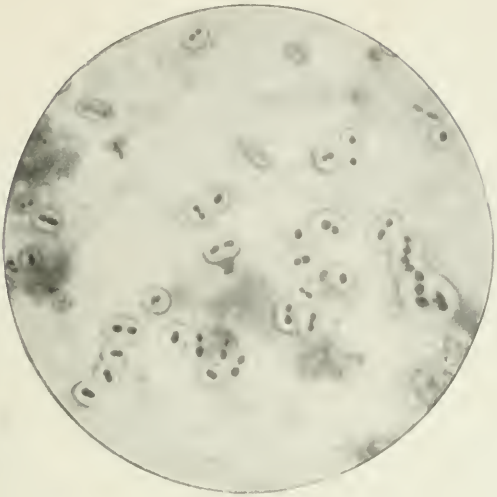


FIG. 1.

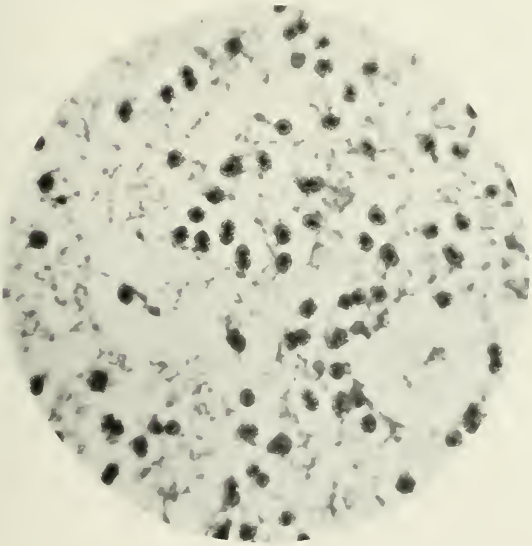


FIG 2.



FIG. 3.



FIG. 4.



FIG. 5.

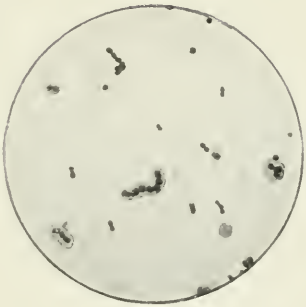


FIG. 6.

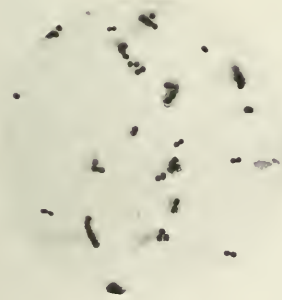


FIG. 7.



FIG. 8.

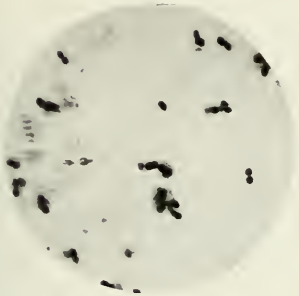


FIG. 9.

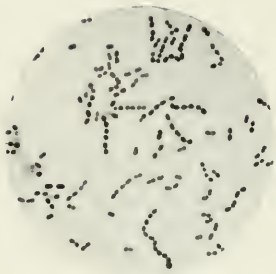


FIG. 10

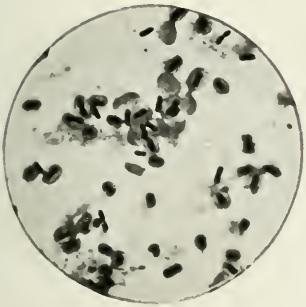


FIG 11.

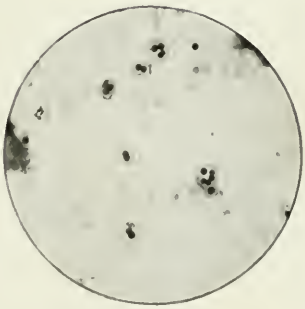


FIG. 12.

STUDIES IN HAEMOLYSIS WITH SPECIAL REFERENCE TO THE PROPERTIES OF THE BLOOD AND BODY FLUIDS OF HUMAN BEINGS.

By H. T. MARSHALL, M. D., Fellow of the Rockefeller Institute for Medical Research.[1]

(*From the Royal Institute for Experimental Therapy, Frankfort-on-the-Main. Geh. R. Prof. Dr. P. Ehrlich, Director.*)

A. Comparison of the Receptors of the Red Blood-Corpuscles of Man and of the Monkey (*Makakus rhesus,* and *M. cynomolgus*).

B. Behavior of Human Blood-Corpuscles in the Presence of Serum from Various Animals, and the Haemolytic Properties of Normal Human Serum.

C. Haemolytic Properties of Human Transudates and Exudates.

A. COMPARISON OF THE RECEPTORS OF THE RED BLOOD-CORPUSCLES OF MAN AND OF THE MONKEY (*Makakus rhesus,* AND *M. cynomolgus.*)

The comparative study of the body cells of man and of those animals that are zoologically nearly related is not only of great theoretical interest, but also depends upon important practical considerations. Attention has already been called to this point by Ehrlich,[2] who said in his address at Hamburg: "I think it probable that the isotoxins will play a great rôle in diagnosis and pathology. Metschnikoff found that, when he produced a chromic acid nephritis in dogs, an isonephrotoxin developed in their blood serum, as was

[1] Aided by a grant from the Rockefeller Institute for Medical Research.

[2] Ehrlich, die Schutzstoffe des Blutes. Vortrag auf der 73. Versammlung deutscher Naturforscher und Aertzte, Hamburg, 1901. *Deutsche med. Wochenschrift,* 1901, xxvii, 865, 888, 913.

shown by the fact that this serum produced a nephritis when injected into normal dogs. It is more than probable that the most diverse isotoxins may occur in human beings—as has already been demonstrated with certainty in the case of the blood by various authors, among whom are Landsteiner and Ascoli.[3] " With the exception of experiments with the red blood-corpuscles, we can, of course, make no experiments upon the isotoxins of the body cells of man, but there are many indications that it will be possible to perform these experiments upon monkeys and thus to win a new basis for the pathology and therapy also of man."

As the isotoxins are acquiring an ever increasing interest in the study of pathological processes, it seemed profitable to investigate the relations between the receptors of man and monkey, and as the red blood-corpuscles are the cells most readily obtained, they were chosen for the study.

In this investigation the same methods were applicable that were employed by Ehrlich and Morgenroth[4] to demonstrate that there are certain receptors common to the red blood-corpuscles of goat, sheep and ox.

It was to be expected that investigation would reveal a partial correspondence between the receptors of the red blood-corpuscles of man and of the monkey, for it had already been demonstrated by means of the precipitin reaction that monkey serum and human serum contain certain specific substances in common. Thus Wassermann, Uhlenhuth, Stern, and Nuttall, all found that when rabbits were injected with human serum, their serum acquired the property of giving a precipitate when added to human serum. This precipitation was a specific reaction for human serum and occurred with the serum of no other animals except monkeys, and even in the last case the reaction was only slight. This fact shows that a part of the specific substance affected by this precipitin is common to the serum of man and monkey.

[3] See, for example, the recent work of Ascoli and Figari upon nephrolysins, *Berlin. klin. Wochenschr.*, 1902, xxxix, 560, 634.

[4] *Berliner klinische Wochenschrift*, 1900, xxxvii, 453; 1901, xxxviii, 569, 598.

Hitherto there have been no reports of investigations of other haptines of these species. In my experiments the blood and serum were obtained from two monkeys; one, *Makakus cynomolgus,* the other, *Makakus rhesus.* The human blood employed was infant's blood obtained from the maternal end of the umbilical cord. In all cases the amount employed in experiments was 1.0 cubic centimeter of a 5% suspension.

I. Action on the Blood-Corpuscles of Monkey and of Man, of Serum from an Animal Previously Treated with Injections of Human Blood.

The action of sera specific for human blood was tried upon monkey blood. It was found that the inactivated serum of a goat previously treated with human blood contains amboceptors for monkey blood and is reactivated by rabbit serum-complement better than by the complements of guinea-pig serum, human serum or monkey serum. The inactivated serum of a rabbit treated with human blood also contains amboceptors for monkey blood, and is reactivated by the complements of guinea-pig serum better than by rabbit serum, human serum or monkey serum.[5]

Interesting results were obtained from experiments to determine the smallest dissolving dose of amboceptor and of complement for human blood and monkey blood.

In the amboceptor tests, decreasing amounts of inactivated human-blood-rabbit serum were employed with a fixed amount of guinea-pig serum complement, and a fixed amount of monkey blood or human blood: 0.5 cubic centimeter guinea-pig serum was used as complement in the monkey blood experiment; 0.1 cubic centimeter guinea-pig serum as complement in the human blood experiment.

In each case 0.075 cubic centimeter of amboceptor serum was the smallest dose that effected complete solution of the blood-corpuscles.

[5] For convenience the serum of a goat previously treated with human blood corpuscles will be called "human-blood-goat serum." In the same way, specific rabbit's serum will be called "human-blood-rabbit serum," and so in other cases; the name of the material injected will be placed first in the compound word, the name of the immunised animal second.

Complement determinations were then made, using 0.1 cubic centimeter amboceptor serum for each specimen of blood with decreasing amounts of guinea-pig serum complement. The result in this case was that 0.075 cubic centimeter of complement reactivated the human blood and amboceptor, while 0.3 cubic centimeter of complement was required for the reactivation of the amboceptor with the monkey blood.

A similar result was obtained when inactivated human-blood-goat serum with rabbit serum complement was used as haemolysin, the dose of amboceptor being the same for monkey blood and human blood, the dose of complement being twice as great in the case of monkey blood as in the case of human blood.

Abundant experimental proof was obtained showing that the serum of untreated goats and rabbits possessed no such amboceptor for monkey blood as was present in the human-blood-goat and human-blood-rabbit. It was also shown that the human-blood-goat serum and human-blood-rabbit serum had no unusual action upon the blood-corpuscles from animals of other species. *In other words, it was shown that these two sera were specific for human blood, and at the same time had a specially strong action upon the blood of the monkeys employed.*

II. Action on the Blood of Monkey and of Man of Serum from an Animal Previously Treated with Injections of Monkey Blood.

Two rabbits were immunized by treatment with blood-corpuscles of *Makakus rhesus*. As only small amounts of monkey blood were available, the degree of immunity reached was not high. With each rabbit 0.025 cubic centimeter of the inactivated serum exactly sufficed to dissolve 1.0 cubic centimeter of 5 per cent suspension of monkey blood (*M. cynomolgus*), when reactivated with guinea-pig serum. These two monkey-blood-rabbit sera acted upon human blood-corpuscles only slightly more markedly than did the serum from a normal unimmunized rabbit: 0.5 cubic centimeter of the inactivated

serum producing only a "strong"[6] degree of solution of the human blood-corpuscles, when guinea-pig serum was used as complement.

Experiments showed that the haemolytic action of monkey-blood-rabbit serum upon monkey blood was destroyed by subjecting the serum to a temperature of 52° C. for one-half hour, while after one-half hour at 50° C. there was still evidence of haemolytic action.

The serum was inactivated at 52° C. and tested for amboceptor for human blood-corpuscles with a variety of sera as complement. It was found that ox serum reactivated best, that guinea-pig serum and human serum acted fairly well, rabbit serum acted very slightly, and goat serum, sheep serum and horse serum almost not at all. With ox serum as complement, 0.8 cubic centimeter of inactive monkey-blood-rabbit serum was sufficient to dissolve 1.0 cubic centimeter of human blood.

III. Relative Affinity of Human Blood and Monkey Blood for Specific Amboceptors.

An elective absorption experiment was made after the method described by Ehrlich and Morgenroth.[7]

Preliminary experiments were carried out by the method of union in order to determine the simple dissolving dose of amboceptor for human blood-corpuscles and monkey blood-corpuscles (*Makakus rhesus*). Decreasing amounts of inactivated human-blood-goat serum were employed as amboceptor: 0.3 cubic centimeter guinea-pig serum was used as complement in each case.

Amboceptor.	Human blood.	Monkey blood.
0.15 ccm.	complete	complete
0.1 "	"	almost complete
0.05 "	filmy	moderate
0.0 (complement alone)	slight	minimal

[6] The degree of haemolysis that has been effected is judged of by the color of the fluid and the amount of sediment remaining. The following terms will be used to designate the degree of haemolysis. Complete, filmy, translucent, strong, moderate, slight, trace, minimal, 0.

[7] *Berliner klin. Wochenschrift*, 1901, xxxviii, 569.

Four parallel series of tubes were then arranged with graduated amounts of inactivated human-blood-goat serum.

Two series, *A* and *B*, were treated with 1.0 cubic centimeter of 5 per cent suspension of human blood, the other two, *C* and *D*, with 1.0 cubic centimeter 5 per cent suspension of monkey blood.

After staying for one hour at 37.5° C. the corpuscles were removed by centrifugalization and the fluids tested for amboceptor for human blood and monkey blood.

Menstruums *A* and *C* were treated with the sediment of corpuscles obtained by centrifugalizing 1.0 cubic centimeter of 5 per cent suspension of human blood; menstruums *B* and *D* were treated with sediment from monkey blood. In each tube 0.3 cubic centimeter of guinea-pig serum was added as complement.

TABLE I.

Amount of human-blood-goat serum.	*A.* Solution of human blood by human-blood-goat serum, previously treated with human blood	*B.* Solution of monkey blood by human-blood-goat serum, previously treated with human blood.	*C.* Solution of human blood by human-blood-goat serum, previously treated with monkey blood	*D.* Solution of monkey blood by human-blood-goat serum, previously treated with monkey blood
1.5 ccm.	almost complete	strong	complete	slight
1.0 "	filmy	moderate	"	"
0.5 "	strong	slight	"	trace
0.35 "	moderate	trace	almost complete	"
0.25 "	"	"	filmy	minimal
0.15 "	slight	minimal	moderate	"
0.1 "	"	"	"	"
0.05 "	"	"	slight	"
0. " (only complement.)	"	"	"	"

It is seen that human corpuscles absorb much more amboceptor than is needed to produce haemolysis, 15 times the simple fatal dose of amboceptor not being quite enough to dissolve two separate doses of human blood. It is also seen that the human red corpuscles pick up amboceptor otherwise available for monkey blood, since ten times the fatal dose of amboceptor serum after previous treatment with human blood, produced only "strong" solution of the monkey blood.

It is also evident that the monkey blood absorbs much more than the fatal dose of amboceptor, as ten times the simple fatal dose produced only "slight" solution of a second supply of monkey blood. The monkey blood absorbs more amboceptor available for monkey blood than is absorbed by human blood, for in the one case the solution of monkey blood is "slight," in the other "strong." This is merely an indication that this specimen of human blood has not receptors for all the amboceptors that have been set free in the serum of the goat as the result of a long continued course of immunization with different supplies of human blood. The monkey blood possesses receptors which the human blood specimens lack, showing affinities for certain amboceptors present in the immune serum. On the other hand, the monkey blood possesses to only a slight degree the power of removing from the immune serum amboceptor available for the human blood-corpuscles, since three and a half times the complete dose of amboceptor is almost sufficient to supply first the receptors of monkey blood, next those of human blood.

These experiments may now be summarized.

1. Serum specifically haemolytic for the human blood acts with remarkable power upon the blood-corpuscles of *Makakus rhesus* and *Makakus cynomolgus*.

2. In each case the amboceptor doses for the monkey blood and the human blood was the same, or very nearly the same.

3. Rabbit serum specifically haemolytic for monkey blood acts only feebly upon human blood. There is a great difference in its action, according to the complementing serum employed.

4. Human blood-corpuscles readily pick up from the serum specific for human blood-corpuscles amboceptors available for monkey blood, but the human corpuscles do not pick up these amboceptors as completely as the monkey blood-corpuscles themselves do.

5. Monkey blood-corpuscles pick up from the serum specific for human blood some of the specific amboceptors. This power is, however, only moderate.

IV. Action of Normal Sera upon Blood-Corpuscles of Man and Monkey.

The action of various active sera upon monkey's corpuscles (*M. rhesus*) was compared with the action of the same sera upon human corpuscles.

I. Serum from Goat.

Serum.	Monkey.	Human
1.0 ccm.	complete	complete
0.5 "	"	"
0.25 "	"	"
0.15 "	translucent	filmy
0.1 "	trace	strong
0.0 "	0	0

II. Serum from Sheep.

Serum.	Monkey.	Human.
1.0 ccm.	filmy	moderate
0.5 "	translucent	slight
0.25 "	moderate	trace
0.15 "	slight	minimal
0.1 "	trace	0
0.0 "	0	0

III. Serum from Ox.

Serum.	Monkey.	Human.
1.0 ccm.	filmy	moderate
0.5 "	minimal	slight
0.25 "	0	minimal
0.15 "	0	"
0.1 "	0	0
0.0 "	0	0

IV. Serum from Goose.

Serum.	Monkey.	Human.
1.0 ccm.	strong	translucent
0.5 "	slight	moderate
0.25 "	0	slight
0.15 "	0	minimal
0.1 "	0	"
0.0 "	0	0

V. Serum from Rabbit.

Serum.	Monkey	Human
1.0 ccm.	0	moderate
0.5 "	0	slight
0.25 "	0	trace
0.15 "	0	minimal
0.1 "	0	"
0.0 "	0	0

Neither monkey's corpuscles nor human corpuscles were dissolved by serum from man, horse, or guinea-pig.

Summary.

1. Goat, sheep, ox, goose and rabbit serum affect monkey blood to almost the same extent that they do human blood.

2. Human, rabbit and guinea-pig serum fail to produce haemolysis of monkey blood or human blood.

3. Monkey blood and human blood behave very similarly in the presence of normal active haemolysins.

These experiments indicate that there is quite a close relationship between the corpuscles of the human being and those of the two varieties of monkey examined. The conclusion is justified that there is a similarity of the receptor groups of the blood-corpuscles of the human being, *Makakus rhesus* and *Makakus cynomolgus*.

While there is this similarity, however, there are receptor groups in the corpuscles of the monkey which are not present in human corpuscles, and likewise the human corpuscles contain receptor groups not shared with the monkey corpuscles.

The following figure (taken from Ehrlich and Morgenroth) illustrates the relation between the receptors of human blood and monkey blood, as it is shown by the elective absorption experiment.



β represents the receptors common to the corpuscles of monkey and man.

α and γ represent the receptors peculiar to man and monkey respectively.

The partial correspondence between the receptors of the blood-corpuscles of man and monkey makes it probable that a similar rela-

tion exists between the other body cells of these animals, and, as Ehrlich has suggested, we may now be able to study the isotoxins of man by means of animal experimentation upon monkeys.

B. BEHAVIOR OF HUMAN BLOOD-CORPUSCLES IN THE PRESENCE OF SERUM FROM VARIOUS ANIMALS, AND HAEMOLYTIC PROPERTIES OF NORMAL HUMAN SERUM.

Attention is already being turned to the application of cytolytic methods in the investigation of various questions of general medical and biological importance. In view of the developments that have taken place in our ideas of immunity, it seems very important to determine the cytolytic properties of human blood according to the newer methods.

The following study of the haemolytic properties of human serum was made under the influence of this idea. Such a research should give a general idea of the cytolytic action of the serum, and furnishes a small part of the preliminary work that must form the basis of an attempt to apply the haemolytic properties of the serum to diagnosis and to questions of pathological interest.

In the recent literature there have appeared reports of several investigations along the lines here indicated.

E. Neisser and Doering[8] studied the blood from twenty persons in hospital wards, including cases of pneumonia, nephritis, tuberculosis, syphilis and emphysema. The action of the human serum upon rabbit blood was quite constant, 0.1 cubic centimeter to 0.15 cubic centimeter of serum completely dissolving 1.0 cubic centimeter of 5 per cent suspension of rabbit blood, and 0.01 cubic centimeter producing a trace of solution.

The human serum acted with decreasing strength upon suspensions of rabbit, goat, guinea-pig and pigeon blood, in the last case causing only a trace of solution. The serum from these animals showed a similar gradation in the lytic action upon human corpuscles, pigeon serum giving barely a trace of haemolysis, and 1.0 cubic centi-

[8] *Berlin. klin. Wochenschrift*, 1901, xxxviii, 593–595.

meter of rabbit serum completely dissolving the specimen of human blood.

Tests made at 0° C. and by centrifugalization showed that the haemolytic action of human serum on rabbit blood is caused by amboceptor and complement. It was possible to inactivate the human serum by heat (56° C.) and reactivate it with active rabbit or horse serum.

A specimen of human serum passed through the bacterial filter retained its complement undiminished for guinea-pig blood, but suffered loss of strength for rabbit blood, hence human serum must contain more than one kind of complement. By suitable experiments it could be shown that human serum contains separate amboceptors for rabbit and guinea-pig blood.

It was possible partly or completely to destroy the amboceptor by prolonged heat—namely, 56° C. for 45 minutes to one hour.

In contrast to E. Neisser and Doering may be cited the results published by Camus and Pagniez.[9] These investigators examined the serum from over one hundred persons and found that the action of the serum on rabbit blood varied considerably. They concluded from their results that the varying haemolytic action is an expression merely of individual differences and has no pathological value.

The preliminary report of Resinelli[10] is of interest in this connection. He investigated the differences in haemolytic power between the serum of the mother and that of the foetus. He found that the latter has distinctly less haemolytic action than the former upon the blood-corpuscles of rabbit, ox, frog and chicken.

My experiments were made with specimens of blood obtained from a maternity hospital.[11]

Upon cutting the umbilical cord, the blood flowing from the placental end was caught in a clean glass flask, defibrinated by shaking with clean iron filings, and brought to the laboratory, where the spec-

[9] *Société de Biologie*, 1901, 242.

[10] *Congresso della Società italiana di Ostetricia e Guinecologia*. Rome, 1901.

[11] I wish in this place to express my thanks to Dr. Vömel, director of the maternity hospital at Frankfort-on-the-Main, for his kindness in supplying the necessary blood.

imen was centrifugalized and the clear or blood-tinged serum was utilized as soon as possible, the sediment of blood-corpuscles being suspended in a volume of 0.85 per cent sodium chloride solution equal to the amount of serum removed, and kept on ice till needed. From this suspension, a 5 per cent suspension was made with 0.85 per cent salt solution, and 1.0 cubic centimeter of this 5 per cent suspension was the standard amount of blood used in all experiments.

The haemolytic action of various normal sera upon human blood-corpuscles was investigated. The result is recorded in Table II.

Test-tubes were arranged with a constant amount (1.0 cubic centimeter) of 5 per cent suspension of human red blood-corpuscles and decreasing amounts of haemolytic serum (from 1.0 to 0.1 cubic centimeter), and an amount of 0.85 per cent salt solution sufficient to bring the total volume of fluid up to 2.0 cubic centimeters. The several tests were made simultaneously and upon the same specimen of human blood. The test-tubes were kept in a thermostat at 37.5° C. for two hours, being shaken from time to time. They then remained in ice chests (7½° C.) over night. The results were recorded in the morning.

TABLE II.

Amount.	Goat.	Sheep.	Ox.	Horse.	Rabbit.	Guinea-pig.	Goose.	Man.
1.0 ccm.	complete	moderate	moderate	0	moderate	trace	translucent	0
0.5 "	"	slight	slight	0	slight	0	moderate	0
0.25 "	"	trace	minimal	0	trace	0	slight	0
0.15 "	filmy	minimal	minimal	0	minimal	0	minimal	0
0.1 "	strong	0	minimal	0	minimal	0	"	0
0.0 "	0	0	0	0	0	0	0	0

Different specimens of serum from the same species exhibit variations in haemolytic activity; for example, it happened several times that guinea-pig serum was found which exerted a distinct, though weak action upon human blood. Hence many repetitions would be necessary in order to determine the relative haemolytic strength of the sera included in Table II. It is seen, however, that goat, goose, rabbit, ox and sheep serum gave positive lytic action, while human, guinea-

pig and horse serum were negative. In other experiments it was found that eel serum is strongly haemolytic and pig serum negative.

The serum of an animal inoculated with human blood-corpuscles acquires strong lytic powers: 0.06 cubic centimeter of the inactivated specific serum of a rabbit and 0.1 cubic centimeter of the inactivated specific serum of a goat containing sufficient amboceptor to dissolve 1.0 cubic centimeter of human blood snspension; while it proved difficult to produce haemolysis of human blood with inactivated normal goat sernm as amboceptor. Of the latter 0.8 cubic centimeter, using guinea-pig or rabbit complement, gave only slight or moderate solution.[12]

No special endeavor has been made to find variations in the behavior of different specimens of human blood-corpuscles. Judging from the numerous tests made with specific haemolysins and antihaemolysins, the action of the human red blood-corpuscles appears to be remarkably constant.

The haemolytic action of normal, active human serum was determined. 1.0 cubic centimeter of a 5 per cent suspension of the blood of each animal tested was treated with decreasing amounts (0.8 to 0.1 cubic centimeter) of a specimen of human serum, the quantity of fluid in the test tubes being kept constant. The experiments were made simultaneously. There was no solution of the blood-corpuscles of man, ox, goat, sheep, guinea-pig, rat or goose. Rabbit blood was completely dissolved by 0.8 cubic centimeter of serum, while 0.2 cubic centimeter showed no action.

In the course of other experiments controls were made in many cases to determine the action of other specimens of human serum upon human, ox and sheep blood. In all cases the human serum failed to show any lytic action.

In one instance that came under observation, there was a very interesting difference between the action of infant's serum and serum from the mother upon guinea-pig blood. The infant's serum in doses

[12] For the reactivation of normal inactive sera see Sachs. *Berliner klin. Wochenschrift*, 1902, xxxix, 182, 216.

up to 1.0 cubic centimeter failed to dissolve this blood, while 0.25 cubic centimeter of the mother's serum dissolved it readily. Apparently, the adult serum contains a haemolysin that is absent from the infant's serum, a condition hitherto unrecorded. It will require further investigation to determine whether this condition is due to absence of both amboceptor and complement from the infant's serum, or of only one of these constituents.

Max Neisser [13] observed a similar difference between serum from old and young horses, the former normally containing antitetanolysin and antistaphylolysin, substances which are completely or nearly completely absent from the serum of young horses, and G. Müller [14] describes the same condition in regard to the agglutinins of the serum of cattle.

From the experiments above recorded it is seen:

1. That the red blood-corpuscles of the infant are dissolved by the serum of various animals, and that the action of one kind of animal serum differs from that of another kind.

2. That infant's serum possesses but feeble haemolytic properties, acting as it does upon only one species of animal blood, namely, the rabbit's. The lytic action in the instance observed by me was weak—weaker than the action found by Neisser and Doering to be constant. This result may be due to individual differences or it may be the result of a peculiarity in infant's serum corresponding to the condition described by Resinelli. (Op. cit.)

In further experiments the complement strength of human, ox and horse serum was compared.

Preliminary tests showed that horse serum exerted no lytic action on human blood-corpuscles, while only 0.2 cubic centimeter of ox serum could be used, as 0.25 cubic centimeter brought about partial solution of the human blood-corpuscles. In one series of tests 1.0 cubic centimeter of human blood suspension and 0.8 cubic centimeter of inactivated specific goat serum were treated with the three

[13] *Deutsche med. Wochenschr.*, 1900, xxvi, 790.

[14] Müller, *Über Agglutinine Normaler Thiersera*, Inaug. Dissert., 1901.

different complements. In a second series, made simultaneously, inactivated normal goat serum, human blood and complement were brought together. After two hours in the thermostat, the specimens were kept on ice over night and the results were noted in the morning.

TABLE III.

	Complement.		
Amboceptor.	0.2 ccm. Ox.	0.75 ccm. Horse.	0.8 ccm. Man.
1. human-blood-goat serum: 0.8 ccm.	complete	complete	moderate
2. normal goat serum:	moderate	0	slight
3. control: no amboceptor	trace	0	0

About three times the completely dissolving dose of inactivated human-blood-rabbit serum and of inactivated human-blood-goat serum were treated with two specimens of fresh human serum as complement.

0.8 ccm. Human serum.	Human-blood-rabbit.	Human-blood-goat.
I	complete	filmy
II	minimal	translucent

The two sera reactivated the goat serum almost equally well, but there was a great difference in the power of the first and the second to reactivate the rabbit serum.

The complement action of several sera was compared, a constant amount of complement being used with decreasing amounts of amboceptor (inactivated human-blood-rabbit serum).

TABLE IV.

Amboceptor	0.3 ccm. Rabbit.	0.2 ccm. Guinea pig.	0.75 ccm. Horse.	0.8 ccm. Man.
0.2 ccm.	complete	complete	complete	complete
0.15 "	"	"	moderate	"
0.1 "	"	"	slight	translucent
0.06 "	filmy	"	trace	slight
0.04 "	"	filmy	0	trace
0.02 "	translucent	translucent	0	0
0.01 "	moderate	moderate	0	0
0.0 "	0	0	0	0

In this case the reactivating power of human serum was weaker than that of rabbit or guinea-pig serum and was approximately as strong as the serum of the horse.

From the experiments just recorded it is seen:

1. That human serum contains complement which will reactivate an amboceptor specifically haemolytic for human blood-corpuscles.

2. That the amount of complement varies with different specimens of serum.

3. That the complement available for one amboceptor and that available for another amboceptor may vary independently of each other.

The following further experiments were made with human serum complement:

Three fresh specimens of human serum were tested as complement for ox-blood-rabbit serum. A preliminary test with ox-blood-rabbit serum reactivated by guinea-pig serum showed that 0.0005 cubic centimeter sufficed to dissolve completely 1.0 cubic centimeter of 5 per cent suspension of ox blood. With three times this amount complement determinations were made, using decreasing amounts of the three specimens of human serum.

TABLE V.

Amount.	Serum I.	Serum II.	Serum III.
0.5 ccm.	moderate	slight	filmy
0.25 "	slight	trace	translucent
0.1 "	trace	minimal	moderate
0.05 "	minimal	"	slight
0.035 "	"	"	trace

Controls with 0.75 cubic centimeter complement without amboceptor were negative.

The complement action of the first of these specimens was also tried with other amboceptors.

Preliminary determinations, using goat serum as complement, showed that 0.075 cubic centimeter of ox-blood-goat serum, and 0.3 cubic centimeter of sheep-blood-goat serum sufficed to dissolve 1.0 cubic centimeter of ox blood and sheep blood respectively. With guinea-pig serum as complement it was found that 0.075 cubic centimeter of human-blood-goat serum was the simple lytic dose.

TABLE VI.

Amount of complement.	0.1 ccm. Ox-blood-goat serum.	0.5 ccm. Sheep-blood-goat serum.	0.3 ccm. Human-blood-goat serum.
1.0 ccm.	moderate	complete	complete
0.75 "	"	"	"
0.5 "	"	"	"
0.35 "	"	"	filmy
0.25 "	"	"	translucent
0.2 "	slight	"	slight
0.17 "	"	"	trace
0.13 "	"	"	0
0.1 "	"	filmy	0

Controls showed that 1.0 cubic centimeter of complement alone produced no haemolysis.

A similar experiment was made with another specimen of human serum, an excess of amboceptor being employed with decreasing amounts of complement.

TABLE VII.

Human serum.	0.5 ccm. Human-blood-goat serum.	0.2 ccm. Human-blood-rabbit serum.	0.012 ccm. Ox-blood-rabbit serum.	0.3 ccm. Sheep-blood-goat serum.
0.8 ccm.	0	strong	moderate	complete
0.4 "	0	slight	slight	filmy
0.2 "	0	trace	trace	translucent
0.1 "	0	0	minimal	moderate
0 "	0	0	0	0

As experiment with human serum as complement for normal haemolysis resulted as follows:

Preliminary determinations showed that 1.0 cubic centimeter of 5 per cent suspension of guinea-pig blood was completely dissolved by:

0.1 ccm. of active dog serum,
0.25 ccm. of active sheep serum,
1.0 ccm. of active rabbit serum,
0.1 ccm. of active ox serum.

The four sera were inactivated at the lowest inactivating temperature (see Sachs, loc. cit.); and used as amboceptor for guinea-pig blood.

TABLE VIII.

Complement.	0.25 ccm. Dog serum.	0.35 ccm. Sheep serum.	1.0 ccm. Rabbit serum.	0.25 ccm. Ox serum.
0.5 ccm. Human serum	complete	complete	complete	complete

The control with 0.5 cubic centimeter human serum alone had no effect upon guinea-pig blood.

All of the experiments with complement reported above were performed in the usual manner, the amboceptor, complement and blood being mixed in test tubes and kept at 37.5° C. for two hours. The following experiments indicate that this is not a reliable test of the actual complement strength of human serum.

Two amboceptor determinations were made in the usual manner, with fixed quantities of two specimens of human serum as complement, and decreasing amounts of amboceptor (inactive human-blood-goat serum). The result was as follows:

TABLE IX.

Human-blood-goat serum.	0.2 ccm. Human serum "A."	0.3 ccm. Human serum "B."
1.0 ccm.	0	0
0.75 "	0	0
0.5 "	0	complete
0.35 "	trace	"
0.3 "	complete	"
0.25 "	filmy	"
0.2 "	strong	moderate
0.0 "	0	0

In each case haemolysis was absent with large amounts of amboceptor; as the amount of amboceptor decreased the reaction reached a maximum and again decreased with insufficient amboceptor.

In order to determine the complement strength when the inhibiting action of the amboceptor fluid was eliminated, the following experiment was made on the same day and with the same materials: Amboceptor and blood-corpuscles were mixed and after one-half to one hour the fluid was removed by centrifugalizing. The corpuscles now laden with amboceptor were suspended afresh in salt solution. Complement determinations now showed that 0.035 cubic centimeter of either specimen of human serum produced complete solution. Smaller amounts of complement were not tried. 0.3 and 0.35 cubic centimeter of amboceptor were used for the first and second complements respectively.

The following day, using the same specific serum, the same blood and the second complement serum, an amboceptor determination

was made by the same method. Corpuscles and amboceptor were united, freed from serum and suspended in salt solution, as described above: 0.15 cubic centimeter of human serum was used as complement.

TABLE X.

Amboceptor.	Degree of solution.
0.14 ccm.	complete
0.12 "	filmy
0.075 "	very slight
0.05 "	0

From this table it will be seen that the presence of the serum of the immunized animal exerts an inhibiting action upon the complement, and, what is rather remarkable, seems to interfere with its own amboceptor.

The goat from which this serum was derived has been frequently injected with human blood, not completely free from serum, and the specific serum therefore contains, in addition to the specific amboceptor, an anticomplement for human serum. Whether the inhibitive action was caused by an excess of amboceptor these experiments do not show.[15]

Summary.

1. Human serum has complement available for a variety of normal and specific amboceptors.

2. The complement differs in amount in various specimens of human serum.

3. The complement strength of different specimens of human serum for different amboceptors has no definite ratio, and the complements for different amboceptors may vary independently.

4. Inactivated human-blood-goat serum inhibits the reactivation of its own specific amboceptor by human serum.

C. HAEMOLYTIC PROPERTIES OF HUMAN TRANSUDATES AND EXUDATES.

A study was made of the haemolytic properties of several ascitic and pleuritic fluids. In the recent literature but little has appeared

[15] Neisser and Wechsberg, "*Ueber die Wirkungsart bactericider Sera.*" *Münchener med. Wochenschr.*, 1901, xlviii, 697.

on this subject. Strauss and Wolff [16] studied the haemolytic action of a series of transudates and exudates, taking as a standard the power of the fluid to dissolve rabbit blood. They tried to correlate the haemolytic action and the physical-chemical condition of the fluid, and concluded that the haemolytic properties of a fluid depend in part upon the complex structure (albumen content) of the fluid. Their experiments indicated that the complexity of the fluid was only one of the factors upon which the haemolytic power depends.

Seven different fluids were examined:

Fluids I and II (ascites) were obtained from a case of cirrhosis of the liver.

Fluids III and IV (ascites) from cases in which the clinical diagnosis was unknown.

Fluid V (ascites) from a patient with myocarditis.

Fluid VI (ascites) from a patient with carcinoma of the ovaries.

Fluid VII (pleuritis) from a case diagnosed as "tuberculous pleurisy."

Haemolytic Action.

The haemolytic activity of the fluids was tested in the same manner as the human serum, 1.0 cubic centimeter of 5 per cent suspensions of blood from various animals being employed with graduated amounts of the fluid to be examined. Without detailing the experiments, the results are grouped in Table XI, the maximal and minimal degree of solution being noted. The experiments with the various fluids were made at different times.

From a consideration of Table XI it is seen that:

1. All the specimens act as haemolysins for blood from animals of several species.

2. The haemolytic strength of the various fluids differs.

3. The action of several fluids upon the several species of blood can not be expressed by a constant ratio: Fluid "A" dissolves each of two species of blood; fluid "B" dissolves the first species, but not the second.

[16] *Fortschritte der Medizin*, xx, 1, 1902.

TABLE XI.

1 ccm. of 5 per cent suspension of blood.	Fluid I.	Fluid II.	Fluid III.	Fluid VI.	Fluid VII.
Ox.	0.5 ccm. complete. 0.15 " slight.	1.0 ccm. strong. 0.5 " slight.	1.0 ccm. trace. 0.5 " minimal.	1.0 ccm. 0	1.0 ccm. slight. 0.35 " minimal.
Sheep.	0.5 " complete. 0.1 " minimal.	1.0 " moderate. 0.35 " minimal.	0.25 " complete. 0.1 " slight.	1.0 " 0	0.5 " complete. 0.1 " trace.
Guinea-pig.	0.5 " complete. 0.15 " slight.	1.0 " moderate. 0.25 " trace.	0.5 " complete. 0.1 " trace.	0.35 " complete. 0.1 " minimal.	0.35 " complete. 0.1 " slight.
Rabbit.	0.35 " complete. 0.15 " slight.	1.0 " complete. 0.5 " trace.	0.35 " complete. 0.15 " trace.	1.0 " complete. 0.1 " trace.	1.0 " filmy. 0.35 " slight.
Rat.	1.5 " trace. 0.25 " 0	1.0 " minimal. 0.5 " 0	1.0 " minimal. 0.5 " minimal. 0.35 " 0	1.0 " 0	1.0 " slight. 0.5 " 0
Goat.		1.0 " filmy. 0.25 " trace.	0.25 " complete. 0.1 " slight.	1.0 " trace. 0.15 " minimal.	0.25 " complete. 0.1 " moderate.
Goose.	1.5 " minimal. 0.35 " 0	1.0 " minimal. 0.5 " 0	1.0 " minimal. 0.5 " minimal. 0.35 " 0	1.0 " minimal. 0.35 " minimal. 0.25 " 0	1.0 " 0
Chicken.		1.0 " trace. 0.25 " minimal.	1.0 " strong. 0.1 " minimal.	1.0 " slight. 0.15 " minimal.	1.0 " strong. 0.1 " minimal.
Pigeon.		1.0 " moderate. 0.15 " minimal.	1.0 " translucent. 0.1 " minimal.	1.0 " strong. 0.1 " slight.	1.0 " translucent. 0.1 " trace.
Man.	1.5 " 0	1.0 " 0	2.0 " minimal. 1.0 " 0	1.0 " 0	1.0 " 0
Horse.		1.0 " filmy. 0.25 " minimal.	1.0 " filmy. 0.1 " trace.	1.0 " complete. 0.1 " trace.	0.25 " complete. 0.1 " slight.
Pig.		1.0 " minimal. 0.5 " 0	1.0 " minimal. 0.5 " 0	1.0 " 0	1.0 " 0

4. In general, the action of the various fluids, except fluid VI, which acts exceptionally with ox and sheep blood, is very similar.

5. Haemolysis was absent, or of minimal degree, with the blood of man, pig, rat and goose.

6. The lytic action of the fluids is much stronger than the lytic action of the infant human serum examined.

Complete records of the agglutinating action of the fluids were not made. Agglutination was very frequently observed, however. For example, fluid VI agglutinated the blood of the ox, sheep, rabbit, guinea-pig, rat and goose and did not agglutinate the blood of man, chicken, pigeon or goat.

The several fluids exhibited unequal agglutinating powers.

Complement Content.

Complement tests were conducted with the fluids in the manner described when considering human serum, and the results of the tests are grouped in Table XII.

Constant amounts of amboceptor and blood suspension were employed with graduated amounts of the fluid used as complement.

In all cases where the body fluids were tried upon human blood infant's blood was employed.

Experiments made with fluid III gave results like those with human serum complement.

When amboceptor, 0.5 cubic centimeter inactive human-blood-goat serum, suspensions of human blood and complement (ascitic fluid III) were mixed, the complement determination gave the following result:

Complement (ascitic fluid III).	Result.
0.5 ccm.	complete solution
0.35 "	trace
0.3 "	minimal
0.25 "	0

The following day, with the same materials, an amboceptor determination was made. Amboceptor and corpuscles were mixed and after one-half hour the laden corpuscles were recovered by centri-

TABLE XII.

Complement.	Human-blood-goat serum amboceptor with human blood corpuscles.	Ox-blood-goat serum amboceptor with ox blood corpuscles.	Sheep-blood-goat serum amboceptor with sheep blood corpuscles.	Ox-blood-rabbit serum amboceptor with ox blood corpuscles.	Horse-blood-goat serum with horse blood corpuscles.
Fluid I	0.35 ccm. complete. 0.1 " trace. Fluid I alone, 0.		No reactivation.	No reactivation.	
Fluid II		1.0 ccm. translucent. 0.1 " slight. Fluid II alone, 0.	No reactivation.	No reactivation.	
Fluid III	0.1 ccm. complete. 0.02 " slight. Fluid III alone, 0.	No reactivation.		0.1 ccm. complete. 0.012 " 0. 1.0 " (alone) 0	
Fluid VI	0.5 ccm. moderate. 0.35 " 0. Fluid VI alone, 0.	0.2 ccm. moderate. 0.5 " 0. Fluid VI alone, 0.	0.5 ccm. complete. 0.05 " moderate Fluid VI alone, 0.	0.15 " complete. 0.035 " slight. Fluid VI alone, 0.	No reactivation.
Fluid VII	0.5 ccm. filmy. 0.25 " 0. Fluid VII alone, 0.	0.5 ccm. filmy. 0.75 " 0. Fluid VII alone, 0.	0.35 ccm. complete. 0.035 " moderate. 0.5 " fluid VII alone, 0.	0.25 ccm. complete. 0.035 " trace. Fluid VII alone, 0.	No reactivation.

It was found in addition that Fluid II reactivated sheep-blood-rabbit serum and rabbit-blood-goat serum, and did not reactivate inactivated normal dog, sheep or ox serum for rabbit blood. Fluid VI did not reactivate goat or ox serum for rabbit blood, and reactivated both sera very slightly for guinea-pig blood.

fugalization, suspended afresh in salt solution and treated with complement, consisting of 0.5 cubic centimeter of ascitic fluid III.

Amboceptor.	
Human-blood-goat serum.	Degree of solution.
0.1 ccm.	complete
0.075 "	filmy
0.02 "	minimal
control (complement alone)	0

Finding that 0.1 cubic centimeter was the simple completely lytic dose of amboceptor a complement determination was made in the same manner.

Human corpuscles were treated with 0.1 cubic centimeter of amboceptor, the excess of fluid was removed by the centrifugalization, and the laden corpuscles, freshly suspended, were treated with graduated amounts of complement.

Complement (ascitic fluid III).	Degree of solution.
0.1 ccm.	complete
0.075 "	filmy
0.03-0.02 "	slight
0.0 "	0

It is seen here that the presence of the specific serum throughout the reaction makes it necessary to employ five times as much amboceptor and five times as much complement as would be needed were the inhibitive action of the specific serum eliminated.

Experiments described in another article[17] proved that the ascitic fluids exert but very feeble antiamboceptor action upon the human-blood-goat serum, and, therefore, all or very nearly all of the inhibitive action depends upon the amboceptor serum, as in the case considered when dealing with complement of human serum.

Summary.

1. The body-fluids examined contain complements capable of reactivating various amboceptors.

2. The reactivating power is not equal in the several specimens.

[17] Marshall and Morgenroth, *Centralblatt f. Bakteriologie und Parasitenkunde, erste Abtheilung*, 1902, xxxi, 570.

3. The complement strength for a given lysin may vary in several fluids independently of the complement strength for other lysins.

4. In order to determine the actual complement strength of the fluid any inhibiting action of the amboceptor fluid must be eliminated. The same precaution is necessary in order to determine the simple completely lytic dose of amboceptor.

5. The complement action of the body fluids seems more pronounced than that of the human serum examined.

Amboceptor Content.

The several fluids were inactivated by heating to 56° C. for half an hour and examined for amboceptor. No complement action remained after this procedure.

Fluid I was devoid of amboceptor for human red corpuscles.

When ascitic fluid and complement (goat serum or guinea-pig serum) and blood were mixed no amboceptor appeared for ox blood or sheep blood. By the method of union, however, amboceptor was found. Corpuscles and ascitic fluid I were mixed, and after one-half hour the corpuscles were recovered by centrifugalization, washed with salt solution, again centrifugalized, suspended afresh in salt solution and treated with 0.15 cubic centimeter guinea-pig complement.

	Degree of Solution.	
Amboceptor (ascitic fluid I).	Ox blood.	Sheep's blood.
1.0 ccm.	complete	complete
0.5 "	"	moderate
0.25 "	"	slight
0.1 "	filmy	trace
0.0 "	0	"
(complement alone)		

On the same day and with the same materials the following experiment was performed:

Amboceptor for ox blood was removed by treating ascitic fluid I with ox blood-corpuscles freed from serum by several washings with salt solution.

Ascitic fluid thus prepared was recovered by centrifugalization and tested for amboceptor for sheep blood, controls showing that this fluid no longer contained amboceptor for ox blood.

As complement 0.15 cubic centimeter of guinea-pig serum was employed.

Ascitic fluid.	Degree of solution of sheep's blood.
1.0 ccm.	moderate
0.5 "	slight
0.25 "	trace
0.1 "	"
0.0 "	"
(complement alone).	

Summary.

1. Ascitic fluid I contains no amboceptor available for human blood-corpuscles.

2. Ascitic fluid I contains amboceptor for sheep's blood and ox blood.

3. The inhibitive action of ascitic fluid I conceals the presence of the amboceptor when guinea-pig serum or goat serum is used as complement.[18]

4. There are at least two groups of amboceptors in ascitic fluid I. One with affinity for ox blood and one for sheep blood. (In removing the amboceptor for ox blood the ascitic fluid is somewhat diluted with salt solution. The weakened amboceptor action seen in the second sheep blood experiment may be due to this dilution alone; or it may be due in part to a third amboceptor group with affinities for both ox blood and sheep blood.)

An experiment made with ascitic fluid III may be recorded at this point.

Tests were made to learn at what temperature the haemolytic action of fresh active ascitic fluid III occurs; and to determine whether there is union of corpuscle and haemolysin at temperatures too low for haemolysis to occur.

Tubes containing ascitic fluid III and rabbit blood were kept at various temperatures, and after two hours the degree of solution was noted. The corpuscles and fluid were separated by centrifugalization, the corpuscles suspended afresh in salt solution and to the

[18] Marshall and Morgenroth, loc. cit.

menstruum was added the sediment obtained by centrifugalizing 1.0 cubic centimeter of rabbit blood suspension. The tubes were then kept for two hours in the thermostat, and remained on ice over night. The results were recorded in the morning.

Preliminary determinations showed that 0.35 cubic centimeter exactly dissolves 1.0 cubic centimeter of 5 per cent suspension of rabbit blood. 2.0 cubic centimeters of ascitic fluid III and 1.0 cubic centimeter rabbit blood were employed.

TABLE XIII.

Temperature.	After 2 hours.	Menstruum.	Sediment.
5° C.	0	complete	0
10° C.	0	"	0
15° C.	trace	"	0
20° to 38° C.	complete		

This experiment shows that:

1. With ascitic fluid III haemolysis of rabbit blood occurs between the temperature 15° and 20° C.

2. Both amboceptor and complement of ascitic fluid III require a minimal temperature of from 15° to 20° C. in order for union to occur with rabbit blood.

3. Amboceptor and complement of ascitic fluid VII can not be separated by this procedure.

For the sake of brevity the experiments with the various fluids upon blood of man, ox and sheep are tabulated.

The experiments were made by the method of union, using guinea-pig serum as complement. The amount of ascitic fluid used is noted in the table.

TABLE XIV.

Fluid.	Degree of solution of human blood.	Degree of solution of ox blood.	Degree of solution of sheep's blood.
I	1.0 ccm. = 0	0.25 ccm. complete	1.0 ccm. complete
II	1.0 " = 0	1.0 " slight	1.0 " translucent
III		1.0 " trace	0.1 " complete
IV	2.0 " = 0		1.0 " moderate
VI	2.0 " = 0	2.0 " complete	1.0 " complete
VII	2.0 " = 0	0.5 " "	0.25 " "
			lowest complete dose not reached

Controls made without removing the ascitic fluids by centrifugalization gave, for the most part, no solution, as was described for fluid I; but with fluids II, III, V and VII an amboceptor for sheep blood appeared when the fluid, sheep's blood and guinea-pig complement were simply mixed and allowed to stand in the thermostat. The same result occurred with fluid VII and ox blood. In all of these cases much stronger amboceptor action was obtained by the method of union.

Other amboceptor experiments will be given briefly:

Fluid II failed to show amboceptor for human blood when reactivated by fresh ox, goat, sheep or rabbit serum. With fluid II and ox blood the rabbit complement acted slightly. The other three sera gave negative results.

Fluid III showed no amboceptor for ox or rabbit blood when ox, goat, or rabbit serum were used as complement.

Fluid V showed no amboceptor for human or ox blood with human serum as complement.

Fluid VI showed no amboceptor for chicken or goose blood with guinea-pig, ox, goat or rabbit serum as complement.

Fluids VI and VII were tried as amboceptor for several bloods, with guinea-pig serum as complement.

TABLE XV.

Blood.	Fluid VI.		Fluid VII.	
Rabbit	1.0 ccm.	minimal	1.0 ccm.	filmy
Goat	0.25 "	complete	0.25 "	complete
Horse	2.0 "	0	2.0 "	translucent
Guinea-pig	2.0 "	0	2.0 "	slight

Smaller doses of fluid were not employed.

In all cases examined (fluids II, III, IV, VI) it was found that amboceptor for sheep blood was not removed when amboceptor available for ox blood was removed.

From these experiments it is seen that:

1. The pathologic body fluids contain amboceptor for a variety of bloods.

2. The amboceptor content of several fluids for a given variety of blood may vary independently of the amboceptor content for another variety.

3. There is more than one group of amboceptors in the fluids.

4. Different fresh sera act in different degrees as complements for the fluids.

5. No amboceptor was found available for human blood.

EPITHELIOMA ADAMANTINUM.

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An effort is made to include here the more important literature of the subject of this article appearing since 1884. Many cases are reported under titles that make them difficult to find. Others are not described with sufficient accuracy for their certain recognition. The description of the paradental epithelial débris (débris épithéliaux paradentaires) and the pointing out of its probable relation to these tumors by Malassez (1) in 1885 gave to their pathology a more logical and firmer basis than it had previously possessed. Little has been added to knowledge of the subject since that time. The most notable contribution is perhaps that of Chîbret (2), who described the formation of enamel and of cemento-dental tissue in one of these tumors. In 1897 there was published by Goebel (3) a review of the literature on all tumors of the jaw bones referable to the dental system. The majority of the contributions appearing before 1885 are, to a considerable extent, only of historical value. The statistics here presented are based upon twenty-two cases. A brief synopsis of these cases is appended. I have had the opportunity of studying the following case.

Abstract from the Clinical History.—The patient, female, aged thirty years, was admitted to St. Joseph's Hospital. Two years and three months ago she first noticed a small, hard tumor near the first bicuspid tooth on the left side of the lower jaw bone. It grew slowly. In childhood there was nothing unusual about her teeth, which were perfectly regular and even up to the time of her present illness. Examination shows that the tumor extends from angle of jaw nearly to the median line.

The dentist of the patient furnished the following history. Two years and three months ago the patient consulted him for what she considered to be an abscess in connection with a tooth. The enlargement

was apparent both inside and outside the mouth and was quite painful. As nearly as he remembers the teeth were all intact, the crowns being normal and no fillings having been introduced. The inferior maxillary bone was enlarged in the neighborhood of the first and second molar teeth, both of which were loose. On extracting the second molar no fluid appeared. On extracting the first molar there was a considerable discharge of thin, brown fluid without odor (evidently the contents of a cyst). The roots of the extracted teeth were almost absent. The patient was referred for treatment to St. Joseph's Hospital, where an operation was performed by Dr. Nathan Jacobson. Six months after operation there is no evidence of recurrence.

Abstract from the Pathological Record.—The specimen consists of part of the left lower jaw bone, extending from the angle nearly to the median line. The enlargement begins about 3 cm. anterior to the angle of the jaw and involves the bone for a distance of 7 cm. The tumor measures 7 x 3 cm. It is covered with a thin shell of bone.

There are at my disposal for description a few small blocks of tissue from the tumor fixed in Zenker's fluid and decalcified in five per cent nitric acid. In this tissue the cystic quality of the tumor is evident. The largest cyst seen is 7 mm. in diameter and is empty. Sections are cut in paraffin 6 μ thick, and stained with eosin and methylene blue.

Microscopical Examination.—Many of the sections show the mucous membrane of the mouth and a thin shell of bone and fibrous tissue surrounding the tumor. The layer of bone is interrupted in many places. The areas of bone are partially surrounded by osteoblasts and an occasional osteoclast is seen. The tumor itself consists of a connective-tissue stroma, in which there are alveoli formed by epithelial cells. The appearance under low power is well represented in Borst's (4) plates. The epithelial elements represent the enamel organ and are largely in the stage corresponding to the greatest development of the stratum mucosum (Fig. 1). One or two areas, somewhat removed from the periphery, represent the stage immediately preceding the development of the stratum intermedium and the stratum mucosum (Fig. 2, c). No definite karyokinetic figures are seen here, but some nuclei stain more deeply than others and have a

slightly ragged surface. This is represented at d, Fig. 2, where, apparently, the stratum intermedium is beginning to develop. Intercellular bridges, perhaps corresponding to those of the epithelium of the mucous membrane of the mouth, are seen. The appearance here suggests carcinoma. In many places the stratum mucosum is largely replaced by cysts containing a finely granular material staining with eosin. Anastomosis of the alveoli suggests that the epithelial constituents form a solid framework similar to that which has been shown to exist in carcinoma by means of reconstructed serial sections.

In the large alveoli is seen an external layer of cells, which in some places are cylindrical, in other places cubical (Fig. 1, a). The layer is occasionally invaginated and therefore appears in the section to be situated inside of the alveolus. The cylindrical cells perhaps correspond to the inner epithelial layer of the enamel organ, the cubical cells to the outer epithelial layer. Often, but not regularly, within the external layer are one or more layers of flattened cells, which tend little by little to assume the stellate form and correspond to the stratum intermedium (Fig. 1, b). Occupying most of the interior of the solid alveoli is the most characteristic feature of the tumor, the stratum mucosum, or enamel pulp, consisting of anastomosing stellate cells (Fig. 1, c). When seen under a lower power it might be mistaken for mucoid tissue and, especially when present in large areas, might lead to a diagnosis of myxoma. There are invaginations of the external layer of cells which with the adjacent stroma simulate the "Anlagen" of teeth in their early stages (Fig. 1, a). This gives to the alveoli the appearance of gland tubules in a stroma of mucoid tissue (Fig. 1), especially in places where the invaginated stroma has largely lost its fibrillar character and appears homogeneous (Fig. 1, d). Evidences of karyokinesis are seen in the external layer, and to a less extent in the stratum intermedium.

Various stages in the development of cysts are well seen. They are due evidently to a hyaline and granular degeneration of the stellate cells and to an accumulation of fluid between these cells. The formation of the stratum mucosum is apparently associated with

an accumulation of fluid between the cells, the formation of long processes of the cellular protoplasm, and the gradual disappearance of the intercellular bridges (Fig. 1). No evidence of enamel, dentine, or cement is seen. Chîbret (2) has described the formation both of enamel and of cemento-dental tissue in a similar tumor. The stroma (Fig. 2, a) consists of dense connective tissue in which only a few blood vessels (Fig. 2, bb) are apparent.

The tumors under consideration probably originate from structures described by Malassez (1) as paradental epithelial débris (débris épithéliaux paradentaires). A rational theory of the histogenesis of this class of tumors dates apparently from the publication of his first article. He carefully describes and illustrates these cell masses as they occur in the adult and discusses their histogenesis. He attempts to explain how epithelial tumors may originate in the bodies of the jaw bones at a considerable distance from surface epithelium. In the intra-alveolar tissue surrounding the roots of normal teeth he has found masses of cells, and from a study of the developing jaw he concludes that these masses represent the remains of the dental ridge and some of the epithelial structures originating from it, especially the neck and the outer epithelial layer of the enamel organ. A consideration of the histogenesis of adamantine epithelium suggests the possibility that adamantine tumors may arise from the gingival epithelium and from any of the derivatives of the dental ridge. The cellular masses described by Malassez are distributed in the alveolo-dental ligament from the apices of the roots to the epithelium covering the gums. The "débris" may be present in the marrow spaces of the jaw bones entirely outside the alveolo-dental ligament. This fact furnishes an anatomical basis for the origin of tumors of the jaw bones more or less independent of teeth.

Efforts have been made to associate the etiology with irregularities in the development of the teeth, with inflammatory processes, trauma, etc.; but these conditions are probably secondary rather than primary.

The sex of the individuals affected is stated in eighteen recorded cases, eight occurring in men and ten in women. The age when

the tumor was first noticed could be estimated in sixteen cases. The youngest individual was eight years of age and the oldest, fifty-eight. The greatest number of patients, five, were in the fourth decade. In two other cases the specimens are described as consisting of the jaw bones of adults. It has been thought that these tumors are prone to occur in young individuals, especially during the period of dental development. The description and figure from a case described by Massin (8) as congenital do not give evidence that he observed a tumor of this class.

The location of the tumor is mentioned in twenty cases; seventeen times it was situated in the lower jaw, twice in the upper. Becker (5) has called attention to a possible influence of the difference in anatomical relations. In the maxilla perhaps such a tumor might grow into the sinus and not cause any apparent swelling or other prominent disturbances; possibly indefinite neuralgic and other symptoms might be caused in this way.

The situation appears to be as frequent on one side as on the other. In the lower jaw the main mass of the tumor is most frequently at the angle of the jaw. From here it may extend upward to the articular surface and into the coronoid process and ventrally as far as the median line. In one case the tumor is said to have implicated one entire inferior maxilla; twice the location was in the body ventral to the angle and four times its position was median. Of the two cases affecting the upper jaw, both were on the left side and in one of these the sinus had been invaded. In Case No. 13 (see Table) both sinuses were invaded and both sides of the lower jaw bone were implicated. A median position in the upper jaw is not mentioned.

These tumors develop in the interior of the jaw bone which remains as a thin parchment-like covering. At the time of operation they have varied in size from that of a plum to that of the head of a foetus, but are most frequently about the size of a hen's egg. In two cases it is stated that the tumor was easily separable from the surrounding bone, which suggests that it was encapsulated. In four cases no macroscopical cysts were present, the tumors being solid

throughout. Most of the tumors described have been cystic. The cysts may be as large as a hen's egg and usually contain a clear, yellowish, slightly viscid or serous fluid in which cholesterin crystals may be present. Such cysts may give to the tumor an irregular surface. The presence of a bony framework in the tumor is mentioned in twelve cases. Perhaps this framework corresponds to the alveolar process which forms about normally developing teeth. In Case No. 13 fully developed teeth were present.

Of the microscopical appearance of these tumors the clearest description is that given by Kruse (6). He reports three cases representing different stages in the development of the enamel organ. In the individual cases, also, different stages are represented. In the first case the epithelial constituents consist of dendritically branching twigs composed of epithelial cells and forming solid masses, situated in a poorly vascularized stroma. The form and arrangement of the cells is similar to that of the dental ridge in an early stage of development. The tumor therefore corresponds in its structure to an early stage of the "Anlage" of the tooth. Kruse's second case has in part the same structure as the first, but there is more tendency toward the formation of a peripheral layer of cylindrical cells. In some places small cysts are present and there is one macroscopical cyst 2 cm. in diameter. Comparison with a somewhat later stage of the dental "Anlage," where outer and inner enamel epithelium, stratum intermedium and stratum mucosum are present, show, according to the description, that the epithelial twigs of the tumor are in all details like the dental "Anlage," and that the relation of the cells to each other is the same. The third tumor is conspicuously cystic. Some cysts are microscopical in size while the largest is the size of a hen's egg. But the solid parts are microscopically like the first two tumors, presenting solid twigs of polygonal epithelial cells, some with a peripheral layer of cylindrical cells, some with beginning cyst-formation. The tumor consists largely of well developed cysts. The size and the structure of the cysts vary, but in general a definite size corresponds to a definite arrangement of the cells. In the smaller

cysts the wall is lined with low cylindrical epithelium, while the lumen contains a granular hyaline material. The larger cysts are lined by a more or less cubical epithelium and three to four layers of squamous cells. There is then, according to Kruse, a continuous series representing different degrees of differentiation, each of which has certain individual characters and in addition presents transitional stages to the others.

Chibret's (2) work is especially valuable. He describes a case in which there is a pronounced tendency toward the formation of the various tissues of the teeth. The case presents all the stages in the formation of the tooth up to the development of enamel and of cemento-dental tissue. These substances are found at the borders of the most highly differentiated alveoli. The cemento-dental tissue resembles cement on the one hand, since it contains large osteoblasts, and dentine on the other hand, since branching canaliculi are present and vessels are wanting. In very few of the cases described in the literature does there appear to have been represented the epithelial sheath of Hertwig. Perhaps this explains the absence of roots and of characteristic cement and dentine.

A remarkable specimen is described by Hildebrand (7). He observed in the case of a boy nine years old an excessive development of masses of teeth in the interior of the upper and lower jaw bones on both sides. Not only conglomerations of teeth, but also more or less completely isolated teeth were present. The eruption of the teeth appeared to have been entirely irregular. Perhaps the entire epithelial "Anlage" of the teeth had assumed an abnormal function. In the soft tissues "Anlagen" of teeth in all stages of development were represented, together with dendritically branching masses of epithelium. These masses of epithelium are not described in detail, but if they are similar to those in the cases previously cited, the case perhaps belongs to the group under consideration.

The growth of such tumors apparently takes place by the formation of new epithelial twigs from those already present. The microscopical appearances are not always entirely typical. Some areas

may be extremely suggestive of carcinoma, the alveoli being irregular and containing epithelial cells that are only slightly differentiated. Structures closely resembling epithelial pearls are described. Bennecke (9) has described an invasion of the epithelial masses by vascular processes of the surrounding connective tissue. He believes that this process corresponds to a similar one that takes place in the normal enamel organ. The adamantine epitheliomata are relatively benign. I find no instance in which metastases have occurred. Enlargement of the lymph nodes may result from associated inflammatory processes. In one case a diagnosis of abscess was made and the tumor was not recognized until the abscess was opened.

The name "Adamantinoma" is suggested by Borst (4) for these tumors. The term Epithelioma adamantinum is perhaps more accurately descriptive. Where the cystic quality is pronounced the adjective "cysticum" may be added to the name as proposed by Goebel(3).

In some of the cases already cited there has been a history of trauma, such as a blow upon the jaw. Not infrequently it is said that an inflammatory process in connection with a tooth has been present at the onset. With the growth of the tumor there is pain and swelling, although pain is sometimes stated to be absent. Enlargement of the lymph nodes is mentioned only in infected cases and in four cases was said to have been absent. The absence of ulceration of the mucous membrane is emphasized in seven cases. Its presence is mentioned in only two cases and was apparently due to operative procedures or to inflammatory processes in connection with teeth. In the most careful descriptions it is insisted that the tumors have no connection with the epithelium of the mucous membrane. In the region of the tumor the teeth become more or less irregular and to a considerable extent are lost. In Case No. 13 the development of the teeth is said to have been irregular. Fluctuation could be demonstrated in one case. Its demonstration depends upon the presence of a fairly large cyst near the surface of the tumor. In Case No. 13 the teeth in the tumor could be palpated. The presence of parchment-like crepitation is mentioned in five cases, but could per-

Reference.	Sex and Age.	Duration.	Chief Symptoms.	Situation.	Character of Tumor.	Operation.	Result.
1. Albarran. Soc. de biol., 1887, 618, 607.	Male, 63 years.	(Recurrence) 8 years.	Tumor in mouth.	Left maxilla in region of second molar; growing into sinus.	In large part solid with two cysts; bony framework; fibrous and "mucoid" stroma.	Three incisions in 10 years; extirpation; resection.	Recurrence; second recurrence after 18 years; subsequent history unknown.
2. Becker (I). Arch. f. klin. Chir., 1894, xlvii, 52.	Female, 41 years.	3 years.	"Dental periostitis" at onset; infected.	Left; mandibula from incisors to angle.	Irregular surface.	Incision evacuating pus; part of alveolar process removed.	Recurrence after three years.
		(Recurrence) $1^1/_2$ years.			Recurrence 4 cm. in diameter; contains cysts with clear serous fluid; fibrous stroma containing bone.	Resection of left half of mandibula.	Recovery; No recurrence at end of one year.
3. Ibid. (II).	Female, 26 years.	5 years.	Many teeth destroyed; fluctuation; crepitation.	Left angle of mandibula.	One large cyst with thick, solid wall; contains yellowish fluid.	Two incisions in mouth; external incision with removal of cyst wall.	No recurrence after $2^1/_2$ years.
4. Ibid. (III).					Microscopically resembles No. 3.		
5. Ibid. (IV).	Male, 39 years.	6 months.	Teeth destroyed; fistula in mouth.	Molar region and ramus mandibula on left side		Incision partly removing wall of cyst.	Recurrence after six months.
					Recurrence contains one large cyst, containing serous fluid; epithelial pearl-like structures.	Resection of portion mandibula involved.	No recurrence six months after resection.
6. Bonnecke (I).	Adult.		Teeth about tumor few and irregular.	Median part of mandibula, extending farthest to left.	Nodular, 6 cm. in diameter; thin covering of bone; bony framework; numerous large cysts.	Resection.	Not stated.
7. Ibid. (II).	Adult.		Teeth made irregular and loose.	Median part of mandibula.	Thin shell of bone; one small cyst. Cellular stroma but little stratum mucosum. 5 cm. in diameter.	Resection.	Not stated.
8. Borst. Die Lehre von den Geschwülsten, ii, 605.					Cystic; epithelial pearl-like structures without cornification.		

Reference.	Sex and Age.	Duration.	Chief Symptoms.	Situation.	Character of Tumor.	Operation.	Result.
9. Chibret. Arch. de méd. exper., 1894, vi, 278.	Male, 53 years.		Teeth made irregular; pain; crepitation; ulceration of mucous membrane.	Right; mandibula	Large as head of a foetus; cystic; cemento-dental tissue; enamel.	Resection of right half of mandibula.	Recovery; no recurrence after two years.
10. Derujinsky. Wiener klin. Wochenschr., 1890, iii, 775, 795.	Female, 25 years.	4 years.	Crepitation; ulceration of mucous membrane; probably secondary to operation; infection; enlarged cervical lymph nodes.	Left angle and ramus mandibula	Cystic and solid.	Two years ago removal of tumor ("Sarcoma").	Recurrence.
						Resection of left half of mandibula.	Not stated.
11. Haasler. Arch. f. klin. Chir., 1896, liii, 749.	Female, 64 years.	6 years.	Onset with dental abscess; teeth over tumor lost; crepitation.	Median part of mandibula, extending farthest to right.	Cysts filled with pale yellow fluid, slightly viscid containing cholesterin crystals; bony framework; fibrous stroma.	Resection.	Two years without recurrence.
12. Hammer. Virchow's Archiv, cxlii, 503.	Male.	6 years.		Left maxilla.	One large cyst with many smaller; dense, fibrous stroma with bony framework.	Resection of part of left maxilla.	Ten years without recurrence.
13. Hildebrand. Zeitschr. f. Chir., 1890, xxxi, 282; 1893, xxxv.	Male, 9 years.	1 year.	Teeth irregular and more numerous than normal; enlargement of maxillae and both sides of mandibula; teeth palpable through thinned bone.	Mandibula on right side from angle to median line; left side; both sinuses involved.	Sinus on each side filled with masses of developing teeth; about 200 teeth in all. Masses of epithelial cells branching dendritically.	Incision after six months.	
						External incision into both sinuses; apparently incomplete removal.	
						One year later incision on both sides of mandibula.	Recurrence on both sides of mandibula and in right maxilla one and a half years later.
						Repeated.	
14. Kruse (I). Virchow's Archiv, 1891, cxxiv, 137.	Male, 21 years.	10 years.	Dental abscess at onset; varying rate of growth; infected cyst; teeth absent in part over tumor.	Mandibula on right side from 2 cm. below coronoid process to canine tooth.	Nodular; easily separable from bone; microscopic cysts; stroma with bony framework; thin bony shell.	Resection of right half of mandibula.	Recovery.

Reference.	Sex and Age.	Duration.	Chief Symptoms.	Situation.	Character of Tumor.	Operation.	Result.
15. Ibid. (II).	Female, 12 years.	1 year.	Onset with toothache and swelling of bone; teeth in part absent.	Mandibula on right side from 3 mm. below articulation to canine.	Nodular; greatest diameter 8.2 cm.; cyst 2 cm. in diameter; stroma cellular with bony framework; bony shell.	Resection of right half of mandibula.	Recovery.
16. Ibid. (III).	Female, 30 years.	18 years.	Abscess at onset; irregular rate of growth; infection; fistula. Teeth absent; crepitation.	Mandibula on right side from articular surface nearly to median line.	Greatest diameter 13 cm.; nodular, cysts containing tenacious fluid; bony shell.	Resection of right half of mandibula.	Recovery.
7. Malassez (I).	Male, 11 years.	2 years.	Not stated.	Median part of mandibula.	Bony shell and framework; no cysts.	Resection.	Not stated.
18. Ibid. (II).	Male, 60 years.	23 years.	Irregular rate of growth; mucous membrane not connected with tumor.	Entire right body of mandibula.	Thin shell of bone; greatest diameter 8 cm.; many large cysts; bony framework; epithelial pearl-like structures; irregular masses of undifferentiated cells.	Not stated.	Not stated.
19. Monguidi. Correspondenz Blatt f. Zahn ärzte, 1895, xxiv, 17.	Female, 10 years.	1 year.	Swelling of cheek.	Right side of mandibula.	Thin shell of bone which is easily separated.	Excised several times. Resection of right half of mandibula.	Recurrence. Not stated.
20. Nasse. Centralbl. f. Chir., 1896, xvii, Beilage zu No. 25, p. 33.	Female, 41 years.	10 years.		Left side of mandibula.	Numerous small cysts; bony framework.	Resection of left half of mandibula.	Not stated.
21. Prym. Virchow's Archiv, 1897, cxlviii, 93.	Female, 31 years.	6 months.	Pain; swelling.	Right side of mandibula from angle to 3 cm. from spina mentalis and to coronoid process.	Cysts only microscopical; bony framework; cellular, nonvascular stroma.	Resection of right half of mandibula.	Not stated.
22. Stensland.	Female, 30 years.	2 years.	Swelling and pain; roots of teeth absorbed.	Left side of mandibula.	Diameter 7 cm.; thin shell of bone; cystic; stroma fibrous; dense, bony framework.	Resection.	No recurrence after 6 months.

haps have been demonstrated in more as the tumors were all surrounded by a thin shell of bone. In five cases the tumor was infected. There was present either an abscess or a fistula leading into the mouth. One may readily understand how a cyst might become infected as a result of incisions or inflammatory processes in connection with teeth. In three cases variation in the rate of growth of the tumor is mentioned.

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DESCRIPTION OF PLATE.

Fig. 1.—Showing part of an alveolus.

a. External layer of cubical and cylindrical cells, which is in places invaginated and therefore appears to be within the alveolus.

b. Layer of cells corresponding to stratum intermedium.

c. Tissue formed by anastomosing stellate cells, corresponding to stratum mucosum.

d. Invaginated stroma.

e. Blood vessels in invaginated stroma.

Fig. 2.— *a*. Stroma.

b. Blood vessels in stroma.

c. Cells representing that stage which precedes the formation of the stratum intermedium and stratum mucosum.

d. Cells representing the early development of the stratum intermedium. Nuclei are deeply stained and have a ragged surface suggesting karyokinesis.



Fig. 1

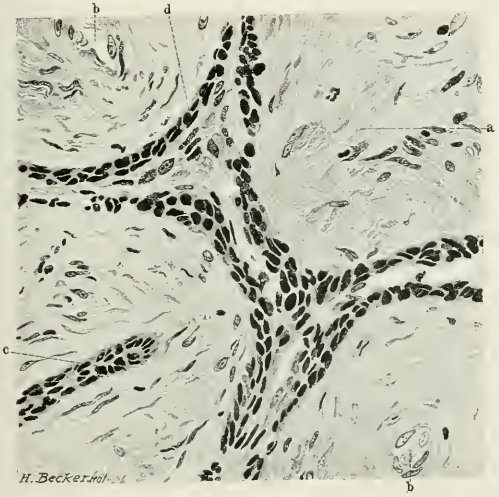


Fig 2

THE BACTERIOLOGY OF BRONCHO- AND LOBULAR PNEUMONIA IN INFANCY.

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The following study of the bacteriology of pneumonia in infancy was carried on at The Babies' Hospital for several years, with the view of determining the relationship between the extent of the pneumonic areas and the variety of bacteria present, and the difference, if any, between the bacteriology of the primary and of the secondary infections.

TECHNIQUE.

Forceps and scalpels were enclosed in sheet iron boxes and sterilized in the hot-air oven for one hour at 120° C.; of these several were kept ready for use. The sternum having been removed with a sterile knife, the lungs were in turn quickly drawn up with forceps, sufficiently to expose the consolidated areas. Incisions or stabs were made into these, a fresh knife being used for each stab. The platinum loop was then plunged into the incision and glucose agar slants inoculated. Cover-glasses were also spread and served as a control. After eighteen to twenty-four hours in the thermostat the tubes were examined and plates prepared from them. All the cultures of pneumococci and many of those of streptococci were inoculated into white mice and rabbits. Cultures from the heart's blood and from the other viscera of the animals which succumbed were made at the autopsy in the same manner as from the human cases. Avoiding the searing of the organs in cases from which the pneumococcus is to be cultivated proved an advantage. Microscopic examination of the lungs was made in every case.

AGE AND SEX.

The one hundred cases ranged from eighteen days to three and a half years of age.

13 were under 3 months old.
26 " between 3 and 6 months old.
30 " " 6 and 12 " "
20 " " 12 and 18 " "
6 " " 18 and 24 " "
4 " " 2 and 3 years old.
1 was 3½ years old.

Among these were 56 males and 44 females.

PRIMARY BRONCHO-PNEUMONIA.

The series includes 33 primary cases, aged from six weeks to two and a half years. In eleven cases the consolidated areas were relatively small and scattered in both lungs; in six they existed in one lung alone. The areas had coalesced, so that the greater part of one lobe was involved in two cases and of two lobes in the same number. In twelve cases the greater part of a lobe was solid while smaller areas were scattered throughout other lobes.

The pneumococcus was present in pure cultures in fifteen, associated with the streptococcus in seven, and the staphylococcus pyogenes aureus in three cases. The streptococcus was found alone in two cases and associated with the staphylococcus pyogenes aureus in two more. The staphylococcus pyogenes aureus was present alone in two cases, associated with the bacillus coli communis in one case, and the streptococcus and oidium albicans in one case.

The following table shows the anatomical distribution of the lesions, the bacteriology, and the condition of the pleura in the primary cases.

Pneumonic Areas.		Pleura.			Pus.				
Scattered.	All or greater part of one or more lobes.	Normal.	Covered with fibrin.	Covered with pus.	In Bronchi.	Pleura.	Pulmonary Abscesses.	Purulent Infiltration.	Bacteriology.
—		—	—	—	—	—	—		Pneumococcus.
—			—	—	—	—	—	—	" and streptococcus.
—			—	—	—	—	—	—	" " staph. pyog. aureus.
—		—		—	—	—	—	—	Staphylococcus pyogenes aureus.
	—	—	—		—		—	—	Pneumococcus.
	—	—	—		—		—		"
	—	—	—		—		—	—	"
	—	—	—		—		—	—	"
	—	—		—	—	—	—	—	"
	—	—		—	—	—	—	—	"
	—	—		—	—	—	—	—	"
	—		—	—	—	—	—	—	"
	—	—			—		—		" and streptococcus.
	—	—		—	—	—	—	—	" " "
	—	—		—	—	—	—	—	" " staph. p. aureus.
	—		—	—		—	—	—	" " " " "
	—	—		—	—	—	—	—	Staphylococcus pyogenes aureus.
	—		—	—	—	—	—	—	Streptococc. and staph. pyog. aureus.
	—	—	—		—		—	—	" " " " "
	—	—		—	—	—	—	—	" , s. p. aureus, soor.
	—		—	—	—	—	—	—	Staphylococcus p. aureus, b. coli com.
			—	—	—	—		—	Pneumococcus.
			—	—	—	—	—		"
			—	—	—	—	—	—	"
		—		—	—	—	—	—	"
		—		—	—	—	—	—	"
		—	—				—	—	"
		—		—				—	" and streptococcus.
		—		—	—	—	—	—	" " "
		—	—		—		—	—	" " "
			—	—	—	—	—	—	" " "
			—	—		—	—	—	Streptococcus.
			—	—	—	—		—	"

— means none.

The relatively large percentage of cases in which the pneumococcus was found, alone or in association with other organisms, may be

attributed to three factors: the short time elapsing between death and autopsy, the use of glucose glycerine agar for the first inoculations, and the avoidance of heating the lung before taking cultures from it.

While the pneumococcus was present in 81 per cent of the cases in which all or the greater part of one lobe was involved, being alone in 41 per cent, it was found in 71 per cent of the focalized cases, in 47 per cent being in pure culture. It would seem, therefore, that in the present primary series the pneumococcus was present in a somewhat larger percentage of cases of broncho-pneumonia involving the greater part of one or more lobes, as compared with those cases in which the pneumonic areas were merely scattered throughout one or both lungs. In the fifteen cases due to the pneumococcus alone, there was acute fibrinous pleurisy present in six, acute purulent exudate in five, and a normal pleura in four. Of the twenty-five cases in which the pneumococcus was present, eight presented no lesion of the pleura. Both cases in which the streptococcus was found in pure culture had a healthy pleura. There was a fibrinous pleurisy in both of the cases due to the staphylococcus pyogenes aureus. Three cases were complicated by inflammation of one or more other serous membranes (pericardium, peritoneum, pia mater, synovial membranes) from all of which the pneumococcus was cultivated. One case, due to the streptococcus and the staphylococcus pyogenes aureus, was followed by empyema and osteo-myelitis of one rib. Two cases showed the presence of true lobar pneumonia as well as broncho-pneumonia, and the pneumococcus was present in both lesions.

SECONDARY BRONCHO- AND LOBULAR PNEUMONIA.

I. Secondary to Athrepsia. 20 cases, 17 having been diagnosed before death. In 10 cases the pneumonic areas involved all or the greater part of one or more lobes, and in 10 they were scattered throughout one or both lungs.

The pneumococcus was present in pure culture in 5 cases, together with the staphylococcus pyogenes aureus in 5 cases, the staphylo-

coccus pyogenes albus and with the bacillus coli communis each in one case. The streptococcus was found in pure culture in one case, together with the bacillus coli communis in two cases, the staphylococcus pyogenes aureus and the staphylococcus pyogenes albus each in one case. The staphylococcus pyogenes aureus was found in pure cultures in two cases. The bacillus lactis aerogenes and oidium albicans were found in one case. There was fibrinous pleurisy in four cases, two of which were accompanied by purulent bronchitis and one by purulent infiltration of the lung as well. In the latter case the pneumococcus and the staphylococcus pyogenes aureus were found; in the former, the streptococcus and the bacillus coli communis, which combination of organisms was also present in one other case, while the pneumococcus alone caused the fourth one.

II. Enterocolitis. Among these three cases, all having given symptoms of broncho-pneumonia before death, one was complicated by furunculosis. In this latter case both the pneumococcus and the streptococcus were cultivated from the lungs. Among the rest the pneumococcus was found in pure culture in one case, and the bacillus coli communis and the bacillus proteus vulgaris were grown from the third case. This last case showed scattered pneumonic areas in the right upper lobe only, while in the pneumococcus case the entire left upper lobe was solid and in the other there were focalized areas in both lungs and consolidation of the lingula.

III. Diphtheria. Five cases were studied. The pneumococcus was found in pure culture in one case, the streptococcus in two cases, and the Klebs-Loeffler bacillus in one case. Finally in one case the streptococcus and the Klebs-Loeffler bacillus were present not only in the lungs, but in the kidneys as well. In the last one there had been no symptoms of nephritis during life. Laryngeal diphtheria existed in all the cases, while in three the pseudo-membrane had extended into the trachea, although it was never present in the bronchi.

IV. Measles. Three cases, in two of which the pneumococcus was found in pure culture, were examined. Both showed scattered

foci of consolidation. In one there was a very recent fibrinous pleurisy and gangrenous areas in both lower lobes. In the third case empyema, purulent pericarditis, and small pulmonary abscesses developed, the streptococcus alone being found. It had also been cultivated from the empyemic pus before death in this case.

V. Meningitis. Two cases were studied in which one involved the cerebral membranes only. The pneumococcus and the streptococcus were grown from the lungs, which contained scattered pneumonic areas and consolidation of three-quarters of the right lower lobe, and showed fibrino-purulent pleurisy. The pneumococcus was present alone in the meningeal pus and in the kidneys. The second case followed spina bifida. From the scattered pneumonic areas in both lungs in this case the streptococcus and oidium albicans were grown. The streptococcus in pure culture was found in the pus of the cerebro-spinal meninges, but the soor fungus appeared in the cultures made from both kidneys. Thrush had been present in the mouth, but had disappeared before death.

VI. Cerebral abscess. One case occurred in which the terminal pneumonia was due to a mixture of the pneumococcus and colon bacillus.

VII. Malaria. Two cases in which death was caused by bronchopneumonia. The consolidated foci were scattered throughout both lungs. The staphylococcus pyogenes aureus was cultivated from one case and the bacillus lactis aerogenes from the other. Tertian malarial parasites were found in sections of the lungs and spleen in both cases and of the kidneys in one case.

VIII. Septicaemia. Six cases were studied. In two the pneumococcus and the bacillus pyocyaneus were found in the lungs, the bacillus being present in pure culture in the other viscera as well. The streptococcus alone was found in a case following a surgical operation. The streptococcus and the staphylococcus pyogenes aureus were present in one case in which the sepsis resulted from erysipelas originating in a cervical wound. The streptococcus alone was found in the heart's blood and kidneys. Staphylococcus pyogenes aureus

in pure culture appeared in two cases, one of which followed furunculosis, the same organism having been cultivated from several furuncles during life.

IX. Tuberculosis. Twenty-five cases were studied. Eight of these were of the pneumonic type, running a clinical course like that of acute broncho-pneumonia, the tuberculosis having been wholly unsuspected in three instances, in two of which the tubercles were found to be very recent and scattered; in another case there was a cavity in the right middle lobe. In all but two of these eight cases there was consolidation of one or more lobes, accompanied by acute fibrinous pleurisy in three instances and by purulent pleurisy in one instance.

The pneumococcus was found alone in two cases, in association with the streptococcus and with the staphylococcus pyogenes aureus in two cases each. The streptococcus and the staphylococcus pyogenes aureus were combined in two cases. Tubercle bacilli were found in smears and in sections of the tissue in every instance.

In one child the clinical course was that of athrepsia, the child dying of broncho-pneumonia. At autopsy the lungs contained recent miliary tubercles and scattered areas of broncho-pneumonia, from the latter of which the pneumococcus was grown in pure culture.

Sixteen cases ran a clinical course of general miliary tuberculosis. The lungs showed consolidation of all or nearly all of either one or two lobes in seven cases, in three of which the pneumococcus was present in pure culture. In one case of the focal variety it was also found pure. The pneumococcus and the streptococcus were found in four cases and the pneumococcus and staphylococcus pyogenes aureus in three. The streptococcus was alone in three, the staphylococcus pyogenes aureus in two instances.

In seven of the twenty-five cases smaller or larger cavities were present (cavities of the right lung occurred five times, of the left lung once, of both lungs once). Among these 7 cases the pneumococcus was found in pure culture twice, associated with the streptococcus twice, and with the staphylococcus pyogenes aureus twice. The streptococcus was present in pure culture in one case. In one case of empyema the pus contained tubercle bacilli and streptococci.

Summarizing the above results we find that the pneumococcus was present in 67 of the 100 cases examined. The positive cases can be divided into 25 primary and 42 secondary pneumonias. While the pneumococcus appeared in 76 per cent of the primary cases, it occurred in only 63 per cent of the secondary cases. And while it was present in pure culture in 42 per cent of the primary cases, it was found alone in only 15 per cent of the secondary cases. The following table summarizes the bacteriology of the entire series:

	Primary.	Secondary.	Total.	
Pneumococcus	14	10	24	
" and tubercle bacillus	0	7	7	
" " streptococcus	7	10	17	
" " staph. p. aureus	3	10	13	
" " diphtheria bacillus	0	1	1	67
" " bacillus pyocyaneus	0	2	2	
" " " coli com.	0	2	2	
" " staph. p. albus	0	1	1	
Streptococcus	2	6	8	
" " staph. p. aurens	2	3	5	
" " " " and soor	1	0	1	
" " diphtheria bacillus	0	1	1	
" " staph. p. albus	0	2	2	
" " bacillus coli com	0	1	1	
" " soor	0	2	2	33
Staphylococcus pyogenes aureus	3	6	9	
Staph. p. aureus and bacillus coli com	1	0	1	
Bacillus lactis aerogenes	0	1	1	
" " " and soor	0	1	1	
" coli com. and protens	0	1	1	
	33	67	100	

The 67 cases showing the pneumococcus were accompanied by inflammation of the pleura in 51 per cent (34 cases), the lesion being an acute fibrinous exudate in 22 instances (13 secondary, 9 primary), acute purulent exudate (empyema) in 4 instances (2 primary and 2 secondary), and a fibrino-purulent one in 8 instances (2 primary, 6 secondary). The cases from which the pneumococcus was absent (33) showed pleurisy in one-third of the number (11 cases), the inflammation being of the acute fibrinous variety in 9 instances (3 primary and 6 secondary) and purulent in 2 instances (1 primary and 1 secondary). So that the pneumococcus cases showed a decidedly larger number of examples of pleurisy than did those of other organisms. The percentage of empyema cases were exactly the same (6%) in both varieties.

Purulent infiltrations of the lungs occurred in 7 cases, and all these contained the pneumococcus, either alone or combined with the streptococcus (3 instances) and the staphylococcus pyogenes aureus (1 instance).

Small pulmonary abscesses existed in 7 cases. In 5 of these the pneumococcus occurred, twice combined with the tubercle bacillus, once with B. pyocyaneus and once with the streptococcus. In two instances the streptococcus was present alone.

Of the 100 cases, 45 were coalescent, and therefore "pseudo-lobar" in distribution, with the pneumococcus present in 33 instances, or 73 per cent. In 31 of 55 focalized cases, or 56 per cent, the pneumococcus was also found—a decided decrease.

The cases of primary broncho-pneumonia in the present series show a larger percentage of pneumococcous infections than do the secondary cases. Those cases, both primary and secondary, in which the inflammation involves all or nearly all of a lobe, show a larger percentage of pneumococcus infections than do cases of the focal variety. The pneumococcus cases show a larger percentage of inflammation of the pleura, and a greater tendency to purulent infiltration and abscess formation.

Dürck[1] states that the pneumococcus was present in every case of primary lobar, pseudo-lobar or lobular pneumonia. While our own number of cases containing the pneumococcus is larger than that of Netter[2] and Neumann,[3] it is about the same as Mosny's,[4] whose primary cases, however, numbered only four. Queisner[5] found the pneumococcus in eight cases examined, while Blumer[6] did not find it at all in eighteen.

The secondary cases in the main bear out Pearce's[7] statement that

[1] *Deut. Archiv f. klin. Med.*, 1897, lviii, 368.

[2] *Arch. de med. exp.*, 1892, iv, 28.

[3] *Jahrb. f. Kinderheilk.*, 1899–90, xxx, 233.

[4] Etude sur la broncho-pneumonie, Paris, 1891.

[5] *Jahrb. f. Kinderheilk.*, 1899–90, xxx, 277.

[6] *Albany Med. Annals*, 1901, xxii, 424.

[7] *Boston Med. and Surg. Journ.*, 1897, cxxxvii, 561.

in all cases where a local or general infection existed the associated broncho-pneumonia was due to the same micro-organism. Blumer had the same experience. In the five athrepsia cases in which the pneumococcus was found in pure culture, no other inflammatory lesion was present. The same was true of the one case containing the streptococcus alone. Three cases having the staphylococcus pyogenes aureus in the lungs at autopsy (combined with the pneumococcus in two instances) had shown the same organism in the discharge from a purulent otitis media before death.

Since the influenza bacillus was not searched for as a matter of routine, it may, of course, have been overlooked in some instances. In two cases which came to autopsy with a diagnosis of influenza cultures from the lungs were made on blood agar with negative results.

A MALIGNANT TERATOMA OF THE PERINEUM.

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The clinical history of the case, for which I am indebted to Drs. W. H. Whitehead and I. H. Manning, of Rocky Mount, N. C., is as follows:

The patient was a male child, born in October, 1898. In July, 1899, the child's father noticed a small tumor, about the size of an acorn, situated in the middle line half way between the anus and scrotum. The tumor was subcutaneous, and neither painful nor tender. In October, 1900, it began to increase in size quite rapidly, as the result, the father thought, of several blows upon it; and the penis became persistently erect. The child had no other abnormality, and seemed in excellent physical condition. The growth was removed by Dr. Tiffany, of Baltimore, in February, 1901. There was a speedy recurrence *in loco*, so that under date of March 18, 1901, Dr. Manning wrote me that the site of the wound was occupied by a sloughing cauliflower mass as large as an orange, and that a lymph node in the right groin was the seat of a metastasis, the size of a walnut, ulcerating through the skin.

At the time of the operation two portions of the growth were sent in alcohol to me for examination. One of them, almond-shaped, and about 3 cm. in its longest diameter, consisted largely of bone. Pieces decalcified in 5% nitric acid showed the following structure. Both surfaces of the piece are covered by epidermis, which, though quite thin, can in most places be divided into the three layers characteristic of that structure, with here and there an epithelial peg. The epidermis rests upon a corium which contains rudimentary papillæ, sebaceous follicles, and hair-roots. A little deeper in the section are numerous plates of bone with rather large marrow spaces. In addition, there are found a number of cyst-like spaces lined by an epi-

dermis, which rests upon connective tissue containing sebaceous follicles and hair-roots. These spaces contain amorphous debris and a few hairs—in short, in the interior of the tumor are several minute dermoids. There is another space quite different from those just described. It is lined by mucous membrane, the epithelium of which is composed of stratified columnar ciliated cells. The epithelium presents a sinuous outline, being thrown up into minute ridges by a loose cellular submucosa. Outside of this is a quite distinct circular band of smooth muscle tissue. There is still another space lined by stratified squamous epithelium, which is more or less parallel with the preceding. Indeed, the two run together throughout a considerable extent of the piece, and at one point there is a wide opening between them, with quite a sharp line of demarkation between the two sorts of epithelium. In addition to these structures, there were noted a very few striped muscle cells, and a plate of cartilage which stained deeply with hæmatoxylin. The tumor is fairly well supplied with blood-vessels and lymphatics.

Microscopical examination of the second piece of tissue previously alluded to, shows it to consist of striped muscle tissue extensively infiltrated by masses of new growth. The muscle is, for the most part, in various stages of atrophy and degeneration. Presumably it is a portion of one of the perineal muscles. The tumor masses may be divided roughly, according to size, into large and small. The latter are more or less circular in shape. In some cases it can be made out quite clearly that they are contained within spaces lined by flattened endothelial cells quite distinct from the tumor cells; while in other cases there is no clear line of separation between them, the two passing into each other by a gradual transition. These spaces contain no blood, and are probably lymphatics. In the spaces the cells are cuboidal or spindle-shaped, and have large nuclei, of which some contain well-marked nucleoli, others only chromatic granules. In some of the spaces the arrangement of the cells is distinctly papillary in character: on a blood capillary as a stalk the cells are placed in a thickness of one or more layers; sometimes the

capillary can be traced to a vessel in the wall of the space. The larger masses possess no characteristic shape, and are evidently the result of extensive infiltration of the muscle. It is somewhat remarkable to find the muscle fibers resisting the attack to the extent that frequently muscle fibers, though more or less degenerated, are found within large masses of tumor cells. In these masses the cells are inclined to be spindle-shaped; they are often packed closely together and arranged in columns, separated from one another solely by blood capillaries, which, indeed, constitute the only stroma that the growth possesses. Transitions between the two sets of tumor masses are abundant. A striking feature in both categories is furnished by spaces circular on cross-section and of various sizes, which contain only amorphous granular material and leucocytes. These seem to be due, in some instances at least, to necrosis, in one situation of tumor cells, in another of muscle fibers. In either case the resulting cavity is not filled by tumor cells; on the contrary, the spaces seem permanent, and are lined by tumor cells.

The clinical history of the case and the microscopical examination of the specimens make it certain that we are here dealing with a congenital tumor of a teratoid nature, which has given rise to a malignant neoplasm. Before attempting to proceed to an exact diagnosis let us summarize the leading features. The tumor was found to contain several minute dermoid cysts, plates of bone, and two structures which are susceptible of interpretation as fœtal organs in an exceedingly rudimentary condition, viz., a cylindrical cavity lined by mucous membrane, whose epithelial cells were stratified, columnar and ciliated, surrounded by unstriated muscle tissue; and another cavity communicating with the above, but lined by stratified squamous epithelium. Nothing was seen which could be looked upon as representing the central nervous system. The location of the tumor exactly in the center of the perineum should also be borne in mind.

When we come to consider its exact position among such tumors we are met first of all by the difficulties which arise from the lack of a uniform nomenclature. The classification proposed by the late

Birch-Hirschfeld[1] is commendable by reason of its simplicity. He divides these tumors into the following classes (omitting those congenital mixed tumors which develop from the anlages of the Wolffian body):

A. Tumors developed as the result of invagination and snaring off of portion of the ectoderm or endoderm, or of either of these layers along with mesoderm—dermoid cysts and the so-called entero-cysts.

B. Tumors developed in the same way as above, except that all three layers of the blastoderm are involved. These he calls "teratoids." They contain representatives of all of the germ-layers, but no distinct organs. It might be added that a tumor to be placed in this class should be easily referable to the fœtal anlages in its vicinity.

C. Tumors which are really rudimentary embryos. These are the true teratomata, and may be divided into two varieties: the autochthonous, or the teratomata of the genital glands; and heterochthonous, or those which result from the inclusion of one embryo by another—the cases of *foetus in foetu*.

Following this classification, it seems clear that our tumor belongs either to Class B or to Class C. Birch-Hirschfeld himself, in discussing this classification, admitted that the division between the two classes was more or less artificial, and thought that it might be very difficult to distinguish between them in some cases. It may be said that the present tumor offers such a case. No assistance is to be obtained from a study of similar cases; for, as far as I can learn from an examination of the literature, all the tumors of a teratoid nature which have been found in the perineum belong to Class A of Birch-Hirschfeld's classification, that is, were either dermoids or entero-cysts referable to the changes which occur in the cloaca and urogenital sinus during the development of the external genitals. It is true that in the region dorsal to the rectum numerous teratoid tumors have been discovered, which were quite complex in structure, and which, as a rule, were susceptible of explanation by the theory of invagination at the caudal extremity of the embryo—a region

[1] Allg. patholog. Anat., Leipzig, 1897.

which is remarkable because of the existence of the cloaca and neurenteric canal in addition to a considerable mass of undifferentiated cells. The neurenteric canal in particular is invoked to explain the occurrence of columnar ciliated epithelium in such tumors. It is difficult to understand, however, how such invaginations could produce teratoids anterior to the rectum; and, as has been said, none have been described in that region. It is conceivable, though, that such a tumor as the one under consideration might arise from a partial snaring off of a large portion of the caudal extremity of the embryo at a very early stage, the separated part being included by a regenerated caudal extremity. Such a separated portion would contain anlages to which the various structures contained in the tumor might be referred. The cavity lined by stratified squamous epithelium might be considered as derived from the ectodermal plate which closes the cloaca, and the other cavity might be considered as a representative of the cloaca or, more likely, of one of the genital ducts. The fact that such a great variety of epithelial cells is found in different regions of the urogenital tract, would make it impossible absolutely to deny that the stratified columnar ciliated epithelium found in the tumor originated in that tract. It is a fact, too, that portions of the malignant growth remind one forcibly of the appearances in the fimbriated extremity of the Falloppian tube. It is not impossible that the malignant growth took its origin in the lining of a rudimentary Müller's duct—in which event we might term it a mesothelioma, as the mesothelium furnishes the lining of this duct. The fact that no evidences of a central nervous system were found is not a valid objection to the above theory, as all of the tumor was not at my disposal.

On the whole it would seem simpler to regard this tumor as a true teratoma in the sense employed in Birch-Hirschfeld's classification. If we adopt this view the question of its genesis next arises. The parthogenetic hypothesis, which has received new force from the brilliant experiments of Loeb, could not obtain here, however applicable it may be to teratoma of the genital glands. Accordingly we should have to conclude that the tumor is a true *foetus in*

foetu in a very rudimentary condition, and that the avenue of inclusion was the urogenital sinus, the closure of the genital folds completely covering it in. Under this interpretation the rudimentary organs referred to would represent the anterior portion of the alimentary canal and the respiratory tract, in accordance with the well-known fact that in teratoma the cephalic extremity receives most development. The absence of brain may be explained by the fact that only a portion of the tumor could be examined. However, brain is not found in all teratomata.

That a mass composed of such tissues should give rise to malignant growth does not seem surprising; rather the wonder is that malignant growths do not arise more frequently in connection with teratomata. Montgomery[2] has recently examined the literature with reference to this point, and, omitting certain tumors of the ovary whose nature was obscure, he could collect but ten cases of malignant teratomata.

In the present case a diligent search was made to determine from what tissue of the teratoma the malignant growth took its origin; but nothing was found to shed any light upon this question—possibly because not all of the tumor was at my disposal. The diagnosis, therefore, has to be made as far as possible from the histological structure alone. It would seem most in accordance with this structure to regard the tumor as an endothelioma which, taking its origin in the endothelium of the lymphatics, developed in and along these spaces finally to break through and infiltrate the tissues of the perineum diffusely.

EXPLANATION OF PLATES.

Plate XXVIII.

Fig. 1. Section through middle of the teratoma, *A* small dermoid cyst, *B* cavity lined by stratified squamous epithelium, *C* cavity lined by stratified columnar ciliated epithelium, *D* smooth muscle tissue.

Fig. 2. Tumor masses in lymphatics.

Plate XXIX.

Fig. 3. The same under higher magnification.

Fig. 4. Through the malignant portion of the tumor.

[2] *Journal of Experimental Medicine*, 1898, iii, 259.



FIG. 1.



FIG. 2.

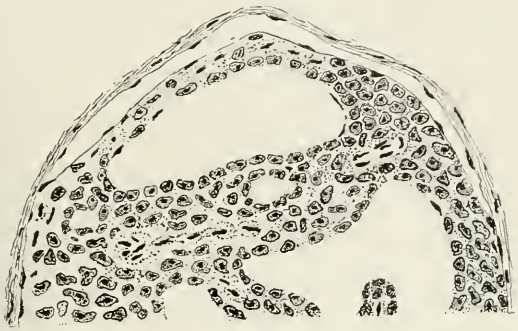


FIG. 3.

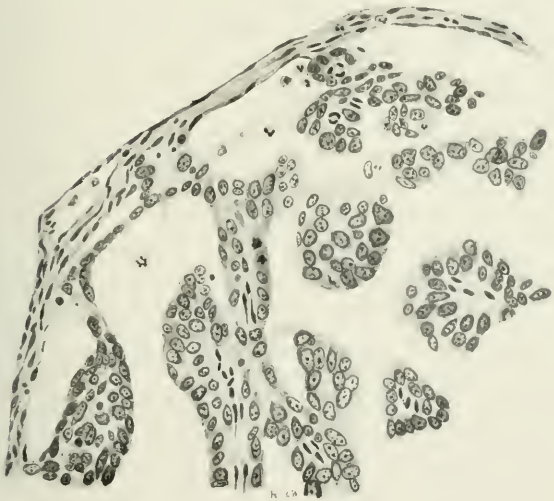


FIG. 4.

DERMATOMYOSITIS, WITH REPORT OF A CASE WHICH PRESENTED A RARE MUSCLE ANOMALY BUT ONCE DESCRIBED IN MAN.

By WALTER RALPH STEINER, A. M., M. D., Hartford, Conn.
(Formerly Medical House Officer, The Johns Hopkins Hospital.)

Inflammatory changes in muscles have long been recognized as occurring independently or in connection with certain diseases. Hunter (1) is said to have described cases of myositis in 1784 and to have noted the insusceptibility of muscular tissue to inflammation and its consequences. I have been unable to find any such reference in his writings. During the past century cases have been published in many different countries and a considerable amount of literature is to be found on this affection.

A multiple muscle inflammation, however, presenting also other well known symptoms, was not described as such until 1887. In that year E. Wagner (2) of Leipsic, Unverricht (3) of the Polyclinic at Jena, and Hepp (4) of Kussmaul's clinic at Strasburg, almost simultaneously reported cases. Though Wagner's case was first published, it was in reality the last seen, for Hepp made his observation two months previously and Unverricht's case had been noted seven years before either of the above two. The pathological findings, with a meager history of this last case, were presented by Marchand (5) to the Schlesische Gesellschaft für vaterländ. Cultur on October 22, 1880, and later appeared in the society's transactions and in the Breslauer ärztliche Zeitschrift of that same year. The case has caused a good deal of confusion among writers on this disease. Herrick (6), Jacoby (7), and others have described it as another case of an earlier observation, while Lewy (8) thinks it is an additional one which Unverricht has later more fully reported. This error may be due to the fact that Unverricht in two of his articles

made the date of seeing the patient a year later than it should have been. Yet a careful examination will convince one that the same case is dealt with. Hepp's case was also reported by Henry Jackson (9) of Boston in 1887—a fact which seems to have been entirely overlooked.

Before these observations we find two others which, although representing cases rather meagerly described and otherwise diagnosed, should undoubtedly be considered as bearing on this disease. I refer to a case seen by E. Wagner (10) in 1863, and to a later one observed by Potain (11) in Paris. They were diagnosed at the time as instances of periostitis and an atypical form of glanders. Following the publication of the first three recognized instances Jacoby (12) and Löwenfeld (13) gave additional cases and discussed the previous papers on the subject. A little later Unverricht (14) presented the clinical history of another case, which differed from those previously reported in terminating favorably. The close association in this patient of dermatitis with a multiple muscle inflammation led Unverricht to coin the term *Dermatomyositis*, which has been frequently adopted to designate this affection. Boeck (15) and Strümpell (16) subsequently published other cases, the latter giving an excellent account of all that was known of the disease.

In the following years many German writers reported so-called cases which were subjected to a critical examination in 1896 by R. Pfeiffer (17), and the histories given by Schnell (18), Lewy (19) (three out of his four cases), Fraenkel (20), Buss (21), Herz (22), Albu (23), Schultze (24), and Schultzen (25) were, rightly we think, adjudged as atypical and belonging to another disease. The cases of v. Kornilow (26), Jollasse (27), and Thiele (28) can be set aside on the same grounds. Neubauer's case (29) more nearly resembles polymyositis haemorrhagica. During these years, however, Fuckel (30), Senator (31), Lewy (32), Köster (33), and Kell (34)[1] have given us undoubted examples of the disease, while Gowers

[1] Two of Kell's three cases have not been added to our list as they were of a doubtful abortive type and somewhat atypical in character.

(35) has reported a case which probably should not be classed here, as it was associated with a polyneuritis. More recently Frohmann (36), Bonnet (37), Lepine (38), Janowsky and Wyssokowicz (39), Bacialli (40), Oppenheim (41), and Christen (42) have added to the number already recorded. Forcheimer's case (43) I have also placed in this list, although it is not as typical as the others. It is interesting to note that one of Oppenheim's cases, reported four years ago as a typical instance of this affection, now presents all the symptoms of scleroderma. During the past two years Vincent (44), Schüller (45) and Jessen (46) have likewise published histories of patients with polymyositis, but none of them had the special type of disease which would entitle them to admission to our group. From time to time different writers have collected the previously existing cases but, with the exception of Lorenz (47) and R. Pfeiffer (48), they appear to have done so without any discrimination. Lewy was able to find twenty-one cases in 1893, while Köster, three years later, added two more to that number. Both of these writers, however, included atypical cases and those of a closely allied affection—polymyositis haemorrhagica.

We have found, including our own, twenty-eight quite typical histories, which are classified according to countries as follows: Germany, 19; France, 2; Sweden, 2; Italy, 1; Cuba, 1; United States, 3; total 28.

Gowers' case is the only one as yet observed in England. In Italy two cases of polymyositis haemorrhagica have been described and one of dermatomyositis, while, though several writers have writen on this affection, the only case yet published in Russia (49)[2] belongs to another type of myositis.

Dermatomyositis may be defined as an acute, subacute, or chronic disease of unknown origin, characterized by a gradual onset with vague and indefinite prodromata, followed by oedema, dermatitis, and a multiple muscle inflammation.

[2] I desire to thank Dr. Thayer for translating this case from the Russian for me.

Etiology.—On this point we are as much in the dark as were the three writers who first described the disease. Hepp and others have spoken strongly in favor of its being due to an infection and the presence of a splenic tumor, fever, and angina, seem to support this view. From Senator's time the following three theories as to its origin have been advanced:

I. It is due to a specific microorganism (vegetable parasite). This view is gradually losing ground. Bacteria have been frequently sought for in the inflammatory oedema and in the tissues, with negative results in every instance save one (Bacialli's case), and cases of this disease reported as being due to certain bacteria have been shown to belong to another class of muscle inflammation. Bauer (50) and Georgievski (51) have recently reported cases of the allied affection—polymyositis haemorrhagica—which were probably due to Staphylococcus pyogenes aureus and Staphylococcus pyogenes albus, obtained in pure cultures from the tissues. In Bacialli's case, however, the Staphylococcus pyogenes albus was said to be very much attenuated in virulence. His findings, as well as Georgievski's, can hardly be considered as conclusive in view of the universal presence of Staphylococcus albus in the deep layers of the skin (Thayer).

II. It is due to an animal parasite. This idea was first advanced by Unverricht, who thought the parasite might belong to the gregarinae. In support of this theory L. Pfeiffer (52) states that the muscle findings in this disease are similar to those seen in the muscles of horses and dogs infected with gregarinae, and he says that Virchow (53) has mentioned cutaneous rashes in hogs that were similar to the changes in the skin occasionally found in dermatomyositis. The only instance, however, in which these parasites were found in man was reported in 1891 by Klebs (54), who observed them in a case of muscular atrophy, where they were at first taken for muscle nuclei. Negative results have attended the search for them in seven cases, in two of which L. Pfeiffer also examined the sections. This unsuccessful quest Pfeiffer would explain as due to the fact that the methods of preserving the tissues and preparing the specimens mili-

tate against a ready recognition of the parasite. They may be present but be overlooked, or, although they can cause a muscle inflammation by their presence or their toxin, the muscles containing them may escape microscopic examination.

III. It is due to a toxin. This view was given prominence by Senator, to whom it was suggested by his second case, which began with symptoms of gastro-intestinal irritation after the patient had eaten stale crabs. Kell's case is also somewhat similar, the symptoms appearing a few hours after the ingestion of fish. No gastro-intestinal symptoms were here noted. In addition to these, Boeck's case has been put in this group, inasmuch as the disease was observed after the energetic rubbing of copaiba balsam into the skin. Boeck, however, does not consider this point to have any etiological significance.

Köster (55) suggests that the symptoms may be due to a primary implication of the vascular system. On this assumption Lepine has proposed the term *Angiomyositis*. It is interesting to note in this connection that Rosenblatt (56) has described a case resembling dermatomyositis, which showed thrombus formation in the vessels, degenerative changes in the vessel wall with fibrin and leucocytes in and about the vessel wall.

Cold and fatigue are said to play a very minor part, though occasionally, as in our case, they seem to be exciting causes.

Distribution.—Cases have been reported, as has been already noted, in the United States and in a number of European countries.

Race.—The Anglo-Germanic has furnished most of the instances, followed by the Latin and Scandinavian. Our patient is the only case in the Negro yet reported.

Season.—The time of year seems to have no connection with the disease. Ten were attacked in the winter, five in the spring, eight in the summer and five in the autumn.

Sex.—The affection has been observed in seventeen males and eleven females. Its distribution among the sexes is probably about equal.

Age.—The disease is usually met with in middle life, though cases at both extremes are found, *e. g.,* Löwenfeld's and Fuckel's cases.

Morbid Anatomy.—Of the seventeen fatal cases autopsies have been performed in all but seven, and in four of these the excision of a piece of muscle was allowed. We have consequently a fair amount of data on which to base our conclusion as to the changes found in this disease.

With the exception of an enlarged and soft spleen, the pathological changes are limited to the muscles. Any or all may be attacked. It was early stated that the muscles of the eye, tongue, heart and diaphragm were exempt, but later investigation has shown that the eye muscles were implicated once (Strümpell's case); the tongue three times (Jacoby's, Strümpell's and Köster's cases); the heart apparently twice (Kell's and one of Oppenheim's cases), and the diaphragm five times (Strümpell's, Köster's, Senator's second case, Wagner's second case, and Janowsky's and Wyssokowicz's case). Batten (57) states that the masseters usually escape. They were implicated in Boeck's, Strümpell's and Unverricht's second case.

The skin covering the muscles is firm and hard on palpation and does not pit on pressure, though oedema is usually present, but may be slight, as in Jacoby's case. On section the subcutaneous tissues present a firm, tense oedema and are usually infiltrated with a yellowish serous fluid.

Macroscopically the muscles may exhibit extensive changes, as Unverricht has shown in an illustration of their gross appearance in his first case. The muscles are swollen, pale red, or pale yellow in color, or may reveal occasionally yellowish gray or diffuse reddish streaks. Hepp considers the muscles to resemble those of the dog. They are often strongly infiltrated with serum and quite moist. In consistency they vary, being hard and firm or soft and boggy. They may be quite friable.[3] They are without lustre and of a dull opaque appearance. Haemorrhages are occasionally seen in them. Microscopically the changes are those of a parenchymatous and interstitial

[3] In Hepp's case the left rectus muscle was found ruptured at the autopsy.

inflammation and may vary in extent, being either focal or diffuse. Again, the different muscle changes may be seen occurring in one and the same fiber. These are frequently separated from one another by the existing oedema or the presence of mononuclear and polymorphonuclear leucocytes. Small haemorrhages may also be seen between them. The fibers themselves are found in all stages of degeneration. They are swollen, coarsely or finely granular, hyaline, or waxy—occasionally fatty. The striae are normal, indistinct, or invisible. Longitudinal or cross cleavage of the fiber has been found, and vacuoles have been described in four instances. In many cases there is an increase in the number of muscle nuclei. Typical interstitial foci of small round cells are found in the perivascular connective tissue and, to a lesser extent, between the muscles. In the subacute and chronic cases the increase in connective tissue may be quite marked in both the perimysium externum and internum, and solitary muscle fibers in the process of degeneration may be seen surrounded on all sides by connective tissue. In five cases the blood-vessels were somewhat dilated and filled with blood.

Wagner has described new muscle formation as taking place in some of the fibers that had undergone waxy degeneration, while Senator has noted an abnormally large number of muscle spindles, two or three being found in each section. Senator, Pfeiffer and Lorenz consider the acute interstitial changes to be primary in this disease, and the degenerative muscle findings to occur chiefly in the second stage. They base this assertion on the reports of the different observers of the pathological processes here met with.

Illustrations of the microscopic changes are given by Wagner (in his second case), Jacoby, Strümpell, and Senator. For the detailed findings described by each writer see the table to be found at the end of this article.

In seven of the autopsies broncho-pneumonia was found as a terminal infection.

Symptoms.—The disease generally attacks persons in the prime of life and in the best of health. Fuckel's case, however, was noted in a

girl after an attack of measles,[4] and both of Wagner's cases had pulmonary tuberculosis, the second one having tuberculous ulcers of the intestines. Senator's second patient was a diabetic.

The onset is almost always gradual with the prodromal symptoms of malaise, weakness, anorexia, or headache. Vomiting was seen in two cases. These symptoms may be of several days' to three weeks' duration, or even longer, as in one case. Occasionally they are absent.

Pain.—Vague rheumatoid pains are next complained of, as well as a stiffness or rigidity in the extremities and back. These pains quickly take on a more definite character and become localized in the muscles. Different muscle groups are then successively attacked. Eventually the whole skeleton musculature may be implicated. Later in the disease the pains are more severe and prevent the patient from making the slightest movement.

Fever is soon noted. It is usually of moderate intensity, and remittent in character in the later stages of the disease. It rarely exceeds 104° F. Just before death it may rise several degrees.

Oedema.—With the fever oedema appears and may implicate the whole body and extremities, the latter presenting at times a most ungainly appearance. It is generally first seen on the face, especially about the eyelids. After the skin inflammation is noticed, it becomes more intense and may remain localized over the affected muscles or spread to surrounding parts. The wrists and ankle-joints are usually spared.

Dermatitis.—Dermatitis is an early symptom and varies greatly in character, being in different cases an erythema, a pseudo-erysipelas, an urticaria, a roseola, or an inflammation resembling erythema nodosum. It may spread continuously or remain limited to the parts where it is first observed. At times it occurs only later in the disease.

Profuse *perspiration* and an enlarged spleen usually accompany the other symptoms.

[4] It is interesting to note that Jessen has but recently reported a case of polymyositis following measles (loc. cit.) It is not typical enough, however, to be classed as one of dermatomyositis.

Nervous System.—No disturbances of sensation are, as a rule, met with, and the nerves are not tender on palpation. The knee-jerks and the electrical reactions are usually either normal or diminished. Lewy got a partial reaction of degeneration in his case.

Stomatitis and *angina* are at times seen, either early or late, in the disease. In Lewy's case and in four of Oppenheim's ulceration of the mucous membranes was observed. On this account the latter has coined the word dermato-mucoso-myositis to more rightly name this disease.

The *urine* is usually normal but may contain albumin.

The course may be characterized by improvement and relapses in the subacute and chronic cases, and atrophy of the muscles may also be here observed. In four cases pigmentation of the skin was found after the dermatitis had disappeared.[5]

Complications.—As stated above, pulmonary tuberculosis, tuberculous ulcers of the intestines, and diabetes are complications that have been met with. Acute haemorrhagic nephritis was observed in Senator's second case, while Jacoby's patient had acute cirrhosis of the liver. In seven instances broncho-pneumonia was found as a terminal infection.

Prognosis.—As all the muscles in the body may be implicated, including those of respiration and deglutition, death may result from suffocation or broncho-pneumonia. Out of the total number of cases (twenty-eight in all) seventeen terminated fatally, so that the prognosis must always be grave. The outcome was fatal for the two patients in the two extremes of life.

Diagnosis.—As a rule no difficulty will be experienced in typical cases. Diseases presenting somewhat similar symptoms are: 1. Trichinosis; 2. Neuromyositis; 3. Infective myositis; and 4. Syphilitic myositis. In the first the initial gastro-intestinal disturbances and the discovery of trichinae in the stools and excised muscle; in 2 the chain of nervous phenomena and the absence of a dermatitis; in 3

[5] For detailed account of the special symptoms, one is referred to Lorenz's excellent and exhaustive article or to Oppenheim's last paper.

the presence of a focus of infection with the bacteriological report on the muscle examination; and in 4 the history of the patient and the objective findings will generally sufficiently differentiate the affection. It is also well to bear in mind that one of Oppenheim's cases turned out, four years later, to be a typical example of scleroderma.

Treatment.—This should be chiefly directed to the relief of pain and to the keeping up of the patient's nutrition. Various analgesiacs have been tried for the former object but no particular drug employed has as yet been found to offer any especial advantage over the others. There is no specific treatment.

From the general observations upon dermatomyositis we now proceed to the detailed consideration of our case.

Case.—John E., aet. 31 years (Medical History No. 9632), laborer, a dark complexioned negro, was admitted to the Johns Hopkins Hospital March 7, 1899, complaining of soreness in his legs, chest, and hands, swelling of his muscles and inability to use them.

Family History.—Unimportant.

Previous History.—The patient had measles and whooping-cough as a child. He also gave a somewhat vague history of malaria four years prior to admission to the hospital. He had had gonorrhœa nine or ten years ago. The attack lasted about seven days; there were no complications. He denied syphilis. He used tobacco sparingly and was a moderate beer drinker.

Work.—Had been working almost constantly since a boy in the stone quarries of Baltimore. His work had been that of a "trencher" and had obliged him to be exposed to all kinds of weather. He had often been drenched through by the snow or rain.

Food.—He had always had a special fondness for beef and pork. Had also eaten a good deal of sausage, bologna and especially blood-pudding. He would often make a meal off sausage which he would buy uncooked to carry with him to work and eat in that condition for his noon-day meal.

Present Illness.—About six weeks before admission he was obliged to stop work because his limbs were sore and swollen. He also suf-

fered from pain in his chest muscles. The onset of his disease began sometime before this but he did not know the exact date. He first noticed that the sole of his right foot pained him, especially on walking. It seemed also somewhat swollen. From here the pain and swelling travelled up to the calf of that leg, then into the thigh, and finally to the hip. Soon after this—about one week, he thinks—his left leg was similarly affected. From here the pain and swelling went to his chest.

The patient stayed at home for about four weeks, either in bed or sitting up in an easy chair. At the end of this time he went to work again but found he could do nothing, for his arms were very sore and weak. Both arms were then affected like the chest and lower extremities. The pain and swelling began in the right hand and went to the forearm, and arm, successively. The left arm was next similarly affected. He described the pains as a "burning like the toöthache," and said they were mostly present when he moved.

For about a year he had been somewhat troubled with shortness of breath. He had some nausea and several attacks of vomiting during the present illness but none at the onset.

Recently he had been voiding large quantities of urine, sometimes being obliged to get up as many as six times at night to urinate. There was no ardor urinæ, but often a sharp pain during micturition and at times a sudden stoppage of the flow.

He had not had any diarrhoea or abdominal pain.

On admission the temperature was 100.2° F., respirations 18, pulse 52.

Physical Examination.—Patient was a stout, well developed man and lay in bed in dorsal decubitus. His expression was dull and listless. Lips and mucous membranes were of good color; the conjunctivae were muddy, but not jaundiced. Pupils were equal and reacted to light and accommodation. The eye movements were normal and no puffiness was noted about the lids. There was no oedema of the face or body and no implication of the muscles of mastication. No appearance of a dermatitis and no signs of a former luetic erup-

tion. No muscle atrophy detected. No swelling of the nerves. No sensitiveness to pressure of the nerve trunks, and no disturbances of sensation.

Thorax.—The pectoral muscles stood out rather prominently and seemed swollen. Some pain was complained of on palpation. The intercostals were apparently not implicated.

The lungs and heart were practically negative on examination. The pulse was 56 to the minute, regular in force and rhythm, and of good volume and tension. There was slight thickening of the vessel wall.

Abdomen negative. Spleen not palpable.

Extremities.—No oedema noted. Flexion, extension, adduction and abduction slight on account of the pain caused. The calf muscles were held very much contracted, and seemed to be quite swollen. The patient complained of much pain and tenderness in them as well as in the muscles of the thighs, forearms, and arms. In these latter regions the contractures and swellings were not as prominent, except in the triceps. Palpation was unsatisfactory on account of the pain elicited and consequently a rigid examination could not be made. The muscles apparently were hard and firm. When the patient lay quiet and motionless in his bed but slight pain was complained of. Marked lineæ atrophicae were seen on the flexor surfaces of both arms, especially about the elbow-joints. The grasps of both hands were feeble and the act was attended with pain. The dynamometer did not register in the right hand and only just registered in the left. The knee-jerks were slightly increased. When the patient endeavored to sit up in bed he held his back and legs very rigid. Great difficulty was experienced when he attempted to button his clothes. His gait was extremely unsteady and walking seemed very painful to him.

There was slight general glandular enlargement.

Blood Examination.—Red blood-corpuscles, 5,788,000; white blood-corpuscles, 5250; haemoglobin, 70 per cent.

The differential count was as follows:

Small mononuclears	18.00
Large mononuclears	9.75
Transitionals	1.25
Polymorphonuclears	64.25
Eosinophiles	6.75

The leucocytes were counted daily for about three weeks and averaged about 8000, the highest count being 12,800 on March 12. The red blood-corpuscles remained about the same as at the first examination but the haemoglobin decreased somewhat in percentage. Differential blood-counts were made from time to time and showed but little change. The last count, made two days before the patient's discharge, was as follows:

Small mononuclears	19.00
Transitionals + Large mononuclears	5.00
Polymorphonuclears	69.00
Eosinophiles	7.00

Shortly after his admission, as the suspicion of his having trichinosis was entertained, a small piece of his left gastrocnemius muscle was excised under cocaine anaesthesia. The examination of this teased in salt solution was negative for trichinae. The individual muscle fibers showed marked degeneration. They were studded with minute granules which varied slightly in size and looked like fatty granules. Many of the fibers had lost their transverse striation. There appeared to be a distinct increase in the number of muscle nuclei. The connective tissue was increased in amount and in places showed well-marked cellular infiltration. The further examination of this muscle will be elsewhere described.

After the patient had been in the hospital about two weeks the pain and muscle tenderness subsided, so that he could be up and about the ward. His gait was, however, extremely unsteady and walking was slightly painful. It was only accomplished when he placed his legs

far apart and frequently he had to use a chair to guide himself with. No Romberg symptom was at any time observed.

On March 18 three small nodules were noted on his hands. They were about the size of a split pea and looked somewhat like acne pustules. They were situated: (1) At the junction of the first and second phalanges of the left thumb; (2) in the mid-metacarpal region of the right thumb; and (3) in the mid-metacarpal region of the right little finger. Pain was complained of in them, especially at night. To the touch they were quite hard and firm. One was excised for microscopic examination as will be later mentioned.

The electrical examination made by Dr. Thomas "was difficult on account of the pain which contractions of the muscles caused. A good contraction could be brought out with moderately strong faradic currents applied to the nerves and by direct stimulation of the muscles. When the muscle was stimulated by the faradic current it remained in a continuous tetanic contraction for several seconds. This was not observed when a moderately strong galvanic current was used. When either pole of the galvanic current was placed over the nerve (the ulnar at elbow) and the current gradually increased, pain was complained of and the muscles were thrown into tetanic contraction. This contraction was not confined to the muscles supplied by the nerve but seemed to be due to voluntary effort on account of the pain. In this case it was most marked in the triceps."

On April 8 he complained of a "feeling as if something was crawling down from the elbows of each arm to the fingers."[4] This was especially noted at night. After about eleven days this symptom disappeared and the patient seemed to have better use of the muscles of his arms. The grasp in both hands then, though stronger, was still quite feeble. The pain in the muscles had entirely gone.

The electrical examination, made a few days later, showed no change except that the muscles were no longer thrown into a tetanic contraction by the faradic current. About this time Dr. Osler noticed a distinct myotonic contraction when the patient grasped a round

[4] Noted also in the cases of Hepp and Lewy, as well as in Wagner's second case.

object. The object was grasped but could not be dropped and his fingers fully extended. This was more observable in the right than in the left hand.

From then on till his discharge, on May 2, there was nothing special to record save that his strength was returning rapidly.

The urine on admission was pale amber in color, clear, 1023 in specific gravity, acid in reaction, negative for sugar but contained albumin to the amount of 0.15 per cent. There was no apparent sediment. Microscopically a few hyaline and granular casts were found. The urine gradually cleared up and at the last examination, made just before the patient's discharge, there was only a trace of albumin and no casts were seen.

Temperature on admission was 100.2°. It was normal the next day and so continued till March 12, when, without any apparent reason, it rose to 101.8°. It was normal again after three days and remained so till the patient's discharge (May 2, 1899).

His subsequent movements were lost track of for about a year, for, although he promised to come daily to the dispensary for electrical treatment, after one visit he disappeared. He was not known at the address he gave us.

He was admitted again to the Johns Hopkins Hospital on February 10, 1900, complaining of cough, soreness in his arms and weakness (Medical History No. 10,905).

Present Illness.—After his discharge from the hospital he had remained quite well and was working in a stone quarry, hammering stone, until nine weeks previous. The present attack occurred, he thought, because he got overheated on a warm day. Dizziness with impairment of vision were the first symptoms. These symptoms came on suddenly and obliged him to stop work. Since then he had remained at home and had complained of soreness and weakness of the trunk and extremities, especially of the right arm and of the left arm at the shoulder. He attributed the trouble in the arms to the fact that he used these muscles most and believed the muscles most used became sorest and weakest. Swelling of the muscles accompanied

this soreness and weakness. In addition to the above described symptoms he had suffered considerably from muscular cramps[1]—usually in his right arm. The forearm would be flexed on the arm during these attacks and the fingers partly bent and held close to each other laterally. The cramps generally lasted from ten to fifteen minutes but were sometimes of longer duration. He did not know how frequently they occurred, but they were never felt in both legs or both arms simultaneously. Pain, not of the cramp-like character, was also complained of in the muscles, especially of the right arm. It was worse on moving about, but was sometimes present when he was at rest.

Had had some attacks of vomiting, especially after eating meat or a good deal of food during his present illness. About ten days before entrance to the hospital he had a shaking chill.

Physical Examination.—The patient looked lethargic. Pain was complained of when the pectoral muscles were palpated. They felt very soft and flabby, even during strong contraction, as did the muscles elsewhere. Although the muscular development seemed enormous there was marked loss of muscular power. No oedema was noted; the spleen was not palpable. Myotonia was not present.

Measurements of Arms and Legs in Centimeters.

	Right.	Left.			Right.	Left
Forearm	31.5	31.5	Contracted	Forearm	32.5	32
Arm	34.5	34		Arm	39	37.5
Calf	36.5	34		Calf	39	38
Mid-thigh	57.5	57		Mid-thigh	61	59

Blood Examination.—Red blood-corpuscles, 6,600,000; white blood-corpuscles, 4300; haemoglobin, 80 per cent.

Differential blood-count:

Small mononuclears	32.7
Large mononuclears	7.0
Transitionals	2.0
Polymorphonuclears	53.3
Eosinophiles	5.0

[1] Hepp also has described this in the later stages of the disease.

Electrical Examination by Dr. Thomas showed that the facial nerve responded to a very slight strength of the faradic current on both sides. Direct stimulation of the muscle caused a tetanic contraction which persisted for several seconds and with stronger currents much pain was elicited. With the galvanic current an ordinary contraction was obtained if the current was weak, but a tetanic contraction if the current was of moderate strength. Direct stimulation of the platysma by the faradic current gave a tetanic contraction which lasted for several seconds after the stimulus was removed. The brachial plexus gave with the faradic a tetanic contraction of the muscles supplied by it. This persisted for several seconds after the current was broken. Direct stimulation (faradic) caused tetanus of the portion of the muscle stimulated and the contraction persisted. On stimulating with the galvanic current the right ulnar nerve at the elbow a sharp, quick contraction was obtained which subsided when the current was broken. Repeated, quick galvanic stimulation did not throw the muscles into tetanus, even with a fairly strong current. Direct stimulation of the right external popliteal nerve caused a contraction of the muscles, particularly of that portion under the electrode. This contraction persisted some time after the current was broken. With the galvanic current, stimulation of this nerve caused a sharp, quick contraction, which did not persist. Direct stimulation of the muscles caused a sharp, quick contraction.

Dr. Thomas also noted that when the patient grasped the hand of the observer but little force was used. When told to let go there was some delay. This was also noticed when he was told to grasp an object, squeeze it, and then let go.

On March 2, pain at the back of the neck was first complained of, and difficulty in turning his head was experienced. This soon vanished, however, and the soreness and weakness gradually subsided. The patient left the hospital in good condition March 21, 1900.

On admission the urine showed conditions similar to those found on his first entrance to the hospital. When he was discharged there was only a trace of albumin and no casts were demonstrated.

His temperature varied between normal and 99°. During his stay

a piece of muscle from the left biceps was excised for microscopic examination.

The patient this time also gave a wrong address and we have been unable to locate him.

Microscopic Examination of Excised Muscle.—Portions of the muscle first removed were hardened in alcohol, bichloride of mercury, and Zenker's fluid. Celloidin and paraffin were used as imbedding agents. The sections were mostly stained with haematoxylin, eosin, safranin, congo red, picrocarmine, and van Gieson's fluid. The Gram-Weigert stain failed to show the presence of bacteria in a number of sections examined. No animal parasites were found. In every section some muscle fibers presented a peculiar anomaly. As seen in cross section this consisted in a collection of fibrillae cut transversely and encircled by a band of fibrillae cut longitudinally (Plate XXX, Fig. 1). Instead of one collection of fibrillae three or more bundles of them were sometimes so surrounded (Plate XXXI, Fig. 3). The muscle fiber showing this anomaly could be normal in structure but more frequently signs of degeneration were found, affecting first the centrally placed, transversely cut fibrillae. These would stain more intensely with eosin, but still reveal the structures that go to make up the Cohnheim fields. In subsequent stages this was difficult or impossible to make out. Finally, a mass staining intensely with eosin was seen, presenting the characteristics of waxy degeneration (Plate XXXI, Fig. 3). The surrounding or encircling longitudinally cut bundles were rarely concerned (Plate XXXI, Fig. 3), but if implicated the degeneration would begin with the innermost fibrillae and pass thence out to the periphery. These fibers were never attacked until the whole transversely cut bundle had undergone waxy degeneration. Eventually the whole muscle anomaly could occasionally be seen to have suffered this change. In almost every instance the outer muscle fiber appeared contracted and shrunken away from its sarcolemma sheath.

In longitudinal sections similar changes were seen. The first stage in the degeneration seemed to consist in the contraction of the inner or now longitudinal fibrillae, by which the cross striations were ren-

dered more prominent (Plate XXX, Fig. 2). Then cleavage of these fibrillae would be noted and a subsequent waxy condition of them with a loss, eventually, of their cross striation. The whole anomaly would occasionally show a marked curling or twisting, such as is met with in degenerating muscle fibers (Plate XXX, Fig. 2).

The sarcolemma was generally normal in appearance but sometimes showed the peculiar blebbing or vascular condition (Plate XXXI, Fig. 4) which Hoen (38) has described in some degenerated voluntary muscle fibers of the uvula. This blebbing was somewhat rare and its origin and development could not be traced so well as in Hoen's case. The appearances seemed to correspond to his description except that the granular material in the blebs was only seen in a few instances and it could not be determined whether this came from the disintegration of the fibrillae. The nuclei occupied the center of the bleb, and their frequent absence could probably be accounted for by the plane of the section being cut above or below that containing the nucleus. The more advanced the degeneration the larger the bleb, but no completely destroyed fiber was observed.

The muscle nuclei presented, at times, a normal appearance, but frequently they were increased in number, swollen, vesicular in shape and contained one to two nucleoli with chromatin granules. They were distributed over the whole extent of the fiber and were often found between individual fibrillae of fibers that had undergone waxy degeneration. Where this degeneration was extreme the nuclei also showed changes. They would be of no definite shape, swollen or decreased in size, would stain intensely in haematoxylin, and would assume bizarre positions.

As a rule, these muscle anomalies were somewhat below the average size of the muscle fibers. They were round or oval in shape, and could be found singly, when they were generally larger in size, or in groups of six or more (Plate XXXI, Fig. 3). They were scattered over all the sections examined and seemed to bear no special relation to the muscle spindles or normal muscle fibers.

The muscle not taking part in this anomalous condition showed interesting changes. Many of the degenerations which have been

described in muscles could be seen. The fibers were normal in size, or swollen and oedematous, or atrophied. First parenchymatous changes were noted, as a result of which the striations failed to come out distinctly in staining. Then the different gradations of waxy degeneration could be made out, and, finally, a complete homogeneous mass, with no intimate muscle structure discernible, could be seen. This was especially marked at the cut ends of the fibers and was probably here due to surgical trauma, as Erb (59) and Weber (60) have pointed out. Occasionally, by the teasing of a fiber, a gap was observed, due to the retraction of the contractile substance, which showed waxy degeneration. The sarcolemma sheath enclosed this gap and within were found very fine granules which took the eosin stain. Owing to the lack of simultaneous retraction there could be seen, in places, irregular cross bands of muscle, also waxy in character. At times this waxy condition had entirely disappeared and the thickened sarcolemma sheath alone remained, filled with rather fine, eosin-staining granules (Plate XXXI, Fig. 6), or fibers presenting this change in only a few fibrillae could be seen, the remaining part exhibiting waxy degeneration (Plate XXXI, Fig. 6).

Longitudinal cleavage was observed here as in the fibers of the muscle anomaly (Plate XXXII, Fig. 7). Cross cleavage was also made out, but rather infrequently. It could not be determined whether the cleavage took place in the membrane of Krause or the plane of Hensen (Plate XXXII, Fig. 8).

In rare instances the bursting of a sarcolemma sheath and the spreading out of the contained fibrillae was noticed (Plate XXXII, Fig. 7). The sheath at the point of rupture always showed Hoen's vesicular degeneration, which had evidently weakened it and might account for its rupture during life. The phenomenon, however, might be a purely artificial one.

Fatty degeneration of the muscle was not observed in any of the sections examined.

Vacuolic degeneration was rare. In all the muscles so affected a swelling of the fiber was seen and, at times, a slight loss of the cross striation. The vacuoles were generally round or oval in shape, of

varying sizes, and so situated that their long axes were parallel to the long axes of the fibers. Their borders were sharp and well defined in every case and their contents consisted of a coarsely or finely granular material containing occasionally two or three nuclei (Plate XXXI, Fig. 5). At times the vacuoles occupied the periphery of the fiber, and then, as a rule, contained a nucleus. Vacuoles occasionally were present in such numbers that the fiber, on cross section, appeared riddled with them. As Schaefer (61) has described, their appearance seemed to be heralded by a round or oval, opaque, non-staining area in a muscle fiber.

In one place there was a considerable extravasation of blood between the fibers. The sarcolemma sheath showed the vesicular degeneration, as above described, rather infrequently. Occasionally the sheath was considerably thickened, especially if its fiber had been completely destroyed.

The nuclei in an unchanged fiber were normal, but with commencing degeneration they would greatly increase in numbers and occupy the center as well as the periphery of the fiber. By a still further increase in number they sometimes almost hid the structure of the muscle. They were oval or vesicular in shape and contained one to two nucleoli. Their long axes were, as a rule, parallel to those of the fiber. At times the nuclei would undergo degeneration, become shrivelled up, stain intensely and lie at right angles to the long axes of the fiber. They disappeared apparently by karyolysis. In no place where there was this proliferation of nuclei were new muscle fibers to be found, but occasionally distinctly reparative processes were observed. The nuclei would lengthen out and become like a rod (Plate XXXIII, Fig. 10). In these cases two or three nucleoli would be seen in them and indentations would indicate a commencing division. Finally, one of these rods would form fourteen or more nuclei (Plate XXXIII, Fig. 11). The next step was the taking on of protoplasm by these nuclei and their formation into myoblasts or sarcoblasts. They received this protoplasm at the expense of the contractile substance of the muscle fiber and consequently might be in hollows of the fiber or, exhausting the whole contractile

substance, might simply be enclosed by the sarcolemma sheath (Plate XXXIII, Fig. 12). Up to this point amitosis was alone observed, but now the myoblasts sometimes showed exquisite examples of karyokinesis (Plate XXXIII, Fig. 13). The new nuclei so formed did not appear to make new cells, but probably in a further stage would form the sarcolemma nuclei of a new muscle cell.

The formation of spindle-cells from these myoblasts was seen, but no new muscle fibers were made out. This might be due to the fact that the process had not continued long enough. Muscle regeneration by the longitudinal cleavage of the old fibers and the subsequent budding of the same was also not observed, though longitudinal cleavage of a collection of fibrillae from an old fiber was occasionally met with (Plate XXXII, Fig. 8).

The myoblastic cells, at times, showed degenerative processes. Their nuclei would then stain intensely and finally disappear, leaving the cell protoplasm which would then take on an intense eosin stain. By the total destruction of all these cells the sarcolemma sheath might be so pressed together by the surrounding fibers as to make it difficult of recognition.

Muscle spindles were, at times, noticed in the sections, but there was no increase in their frequency or any pathological change discernible.

Nerves.—The nerves seemed normal in structure, save that in some instances there was an increased number of nuclei in the sheath of Schwann.

Connective Tissue.—The increase in small round cells in the perimysium externum and internum was comparatively infrequent. It could be seen especially in the perivascular connective-tissue space of the former. Much more marked in these situations was the increase in connective tissue, which was more noticeable in the perimysium externum. The tissue was oedematous and very loose in structure. Occasionally it was seen taking the place of a completely degenerated muscle fiber, or a collection of fibers (Plate XXXII, Fig. 8). It was especially rich in newly formed blood-vessels, and where it bordered on a degenerated muscle fiber it could be seen sending some shoots

across the fiber, if longitudinally cut, and, by further development of other blood-vessels and loose connective tissue, the position of the fiber might be completely occupied by these structures. The increase in fat cells was, at times, quite marked (Plate XXXII, Fig. 9). Occasionally the connective tissue underwent a waxy or granular change and an eosin-staining, granular mass might be alone discernible. In some places large multinucleated giant cells [8] were noted in the connective tissue, near degenerated muscle fibers.

The small nodules, referred to in the patient's first history, on sectioning and staining, proved to be small subcutaneous abscesses. There was nothing specially remarkable about them. No bacteria were noted in them by the Gram-Weigert stain.

The muscle excised from the patient's left biceps on his second admission was hardened in alcohol, embedded in celloidin and stained with haematoxylin and eosin. I have been unable to see these sections as they were destroyed during a small-pox epidemic in the ward. Dr. Harris, who was in charge of the patient at that time, has written to me that the muscle anomaly was wanting in these sections, but a distinct myositis was present, which consisted, especially, in a great increase in connective tissue between the fibers.

It seems fair to conclude that the above case was one of dermatomyositis, although it is unfortunate that in the light of our further knowledge we have been unable to question the patient more minutely on some of the symptoms in this disease. The swelling spoken of as present at the onset was probably oedema, which is always associated with dermatomyositis. No oedema was noted on his admission to the hospital. He was not asked as to a skin eruption, but if it consisted of merely an erythema he might easily have overlooked it on account of his color. Further information as to fever and some other points is also lacking. I regret I did not pay any attention to the macroscopic condition of the portion of muscle first removed. Microscopically the findings [9] are identical with those seen in this affection.

I have been unable to find a satisfactory explanation for the muscle

[8] Seen also by Wagner in his second case.

[9] Frohmann describes peculiar findings in his case, but they are too indefinite for us to judge whether or no he observed this anomaly.

anomaly. It has been described in two instances and two explanations have been given for its occurrence. Both, however, seem unlikely and rather fanciful. Bataillon (62) found it in frog larva. He considers it a degenerative process and says it begins by the tearing of certain of the outermost fibrillae of a muscle fiber. These torn fibrillae then circumscribe those centrally located, and are more or less obliquely placed. They run in all directions and are plainly separated from the central bundle. Often they show a more regular arrangement and by cross section may be seen to encircle, as a ring, with longitudinal fibrillae, the transversely cut fiber. The regularity of their cross and radial striations, as well as their staining properties, speaks strongly against this view. J. Schaffer (63), on the other hand, thinks that the muscle is not at all concerned, but that the encircling ring or band belongs to the sarcolemma, which, by a peculiar arrangement, simulates muscle striations. It is generally found in atrophied fibers of which the sarcolemma sheath has ruptured. After the rupture the sheath is retracted and drawn down in folds over the muscle fiber, below the site of rupture. The fiber above this retraction is without its sheath and the fibrillae are here widely spread apart. The successive infoldings of the retracted sarcolemma sheath causes the appearance of the circular striations. Our case, in Figs. 1 and 3, seems to refute this theory, as the ring or band can be plainly seen shrunken away from the surrounding sarcolemma sheath. (Plate XXX, Fig. 2, appears to show that this ring or band is muscle, cut in cross section.) The radial striations may possibly be due, Schaffer thinks, to the effect of the acids used in Flemming's solution—the hardening agent employed. Some of the tissue was hardened in Müller's fluid and similar pictures were seen, due, as Schaffer conjectures, to a contraction caused by the embedding process. The muscle in this case was from the gastrocnemius of a man. The description and plates correspond quite closely to the findings in our case, but some minor differences exist. In the lower types of vertebrates and invertebrates a bundle of transversely cut fibrillae is found, in cross sections of muscle, surrounded by a band of longitudinal fibrillae, which are in turn encircled by sarco-

lemma with a layer of undifferentiated sarcoplasm. Kölliker (64), Rollet (65), and others have described such pictures. Our anomaly, consequently, may be looked upon as a structure reverting to that seen in the lower types. Atavism, however, does not explain, but merely places the question in another phase.

In the other instance found in man it was also seen in the gastrocnemius muscle, so it may be a normal structure of that muscle and have some hitherto unknown function. It is our purpose to study further the histological structure of gastrocnemius muscles to see how frequently this so-called anomaly occurs and if it has any function of its own. It is interesting to note that it was not found in Kell's or one of Oppenheim's cases of dermatomyositis, though the gastrocnemius muscle was examined.

It is not inconceivable that what I have called above a muscle anomaly may be merely the result of pathological changes in the muscles. The blebbing of the sarcolemma, if of pathological occurrence, would doubtless weaken the muscle sheath and possibly a rupture might subsequently ensue. The outermost muscle fibrillae of the fiber, then no longer closely confined, would have a tendency to spread out. If consequently a cross section of this fiber were cut at a point where these fibrillae have begun to spread out a picture similar to the so-called anomaly (see Plate XXX, Fig. 1) would be obtained.

I have, also, been unable to explain why degeneration begins at the center of the anomaly and goes outward towards the periphery. In cross sections the longitudinal band seemed to press the transverse fibrillae very closely, and by this constriction the nutrition of the latter might be interfered with and the final result be degeneration of the centrally placed muscle bundle. I merely offer this as a possible partial explanation.

I am indebted to many friends who have looked over my sections and given helpful suggestions; especially do I desire to thank Dr. Welch for his kindly aid and valuable advice, and Dr. Osler for allowing me to report this case.

I am under obligations to Dr. Wolfe, of Hartford, Conn., for the photomicrographs which accompany this article.

Reporter.	Year.	Sex.	Age.	Occupation.	Onset.	Fever.	Parts Implicated.
1. E. Wagner.	1863	Woman.	43	Servant.	Swelling of both arms.	Yes.	Neck, thorax, abdomen and arms.
2. Potain.	1875	Man.	17	Farmhand.	Malaise, weakness, pains in extremities, and redness of eye-lids.	Yes.	Pharynx, arms and legs.
3. E. Wagner.	1887	Woman.	34	Cook.	Pain in back and sacrum.	Yes.	Pharynx, neck, thorax, diaphragm, arms and legs.
4. Unverricht and Marchand.	1887	Man.	24	Stone-mason.	Dragging pains in arms and legs.	Yes.	Pharynx, neck, thorax, b'k, abdomen, arms and legs.
5. Hepp and Jackson.	1887	Woman.	36		Malaise, weakness and depression.	Yes.	Pharynx, larynx, neck, thorax, back, abd'n, arms and legs.
6. Jacoby.	1888	Man.	38	Machinist.	Feeling of tension in right calf.	Yes.	Tongue, thorax, back, abdomen, arms and legs.
7. Löwenfeld.	1890	Man.	50	Postman.	Pain in left calf.	Yes.	Pharynx, neck, abdomen, arms and legs.
8. Unverricht.	1891	Woman.	39		Swelling of both legs and urticaria.	Yes.	Face, neck, thorax, back, abdomen, arms and legs.
9. Strümpell.	1891	Man.	70	Gardener.	Nausea, vomiting, headache, and general malaise.	Yes.	Face, eyes, tongue, pharynx, diaphragm, arms and legs.
10. Senator.	1891	Man.	50	Baker.	Pain in right leg.	Yes.	Thorax, arms and legs.
11. Senator.	1891	Man.	40	Restaurant-keeper.	Weakness, anorexia, sleeplessness, dragging pain in back and painful stiffness in limbs.	Yes.	Larynx, diaphragm, abdomen, arms and legs.
12. Boeck.	1892	Man.	21	Laborer.	Violent pains in loins and thighs.	Yes.	Face, neck, thorax, abdomen, arms and legs.
13. Fuckel.	1892	Girl.	8		Diarrhoea and slight fever.	Yes.	Arms and legs.
14. Lewy.	1893	Woman.	25		Malaise, cough and anorexia.	Yes.	Thorax, abdomen, arms and legs.
15. Köster.	1895	Woman.	36		Malaise and weakness.	No.	Face, tongue, pharynx larynx, neck, thorax back, abd'n, diaphr'm, arms and legs.
16. Kell.	1896	Man.	23	Sailor.	Pain in right calf.	Yes.	Pharynx, larynx, thorax, arms and legs.
17. Frohmann.	1899	Man.	42	Inspector.	Malaise and pain in thighs.	?	Arms and legs.
18. Lepine and Bonnet.	1901	Man.	59	Carpenter.	Intermittent, dull pains in the head.	No.	Face, back and arms.
19. Janowsky and Wyssokowicz.	1901	Woman.	23	Servant.	Pain in arms.	Yes.	Pharynx, larynx, neck, diaphragm, abd'n, arms, legs and sphincter ani muscle
20. Bacialli.	1902	Man.	39	Clerk.	Vague rheumatoid pains in upper part of body, especially the arms.	Yes.	Pharynx, neck, thorax, arms and legs.
21. Oppenheim.	1903	Man.	38		Malaise, anorexia, chilly sensations.	Yes.	Pharynx, arms and legs.
22. Oppenheim.	1903	Woman.	40		Erythema, fever, pain in neck and arms.	Yes.	Pharynx, neck, arms and legs.
23. Oppenheim.	1903	Woman.	28		Malaise, fever, profuse perspiration.	Yes.	Pharynx, tongue, arms and legs.
24. Oppenheim.	1903	Man.	48	Merchant.	Fever, roseola, pain in extremities.	Yes.	Pharynx, arms and legs.
25. Oppenheim.	1903	Man.	60	Gymnasium instructor.	Angina, pain in extremities, fever.	Yes.	Pharynx, larynx, arms and legs.
26. Christen.	1903	Boy.	10	Student.	Pemphigus-like eruption, vague rheumatoid pains.	Yes.	Face, abdomen, arms and legs.
27. Forcheimer.	1903	Woman.	40		Irritation of skin and mucous membrane, headache, pain in various parts of body.		Thorax and legs.
28. Steiner.	1904	Man.	31	Stone-quarryman.	Pain and swelling in right foot.	Yes.	Thorax, arms and legs.

Skin Changes.	Atrophy.	Duration.	Result.	Complications.	Cause of Death.	Remarks.
Erythema.	No.	19 days.	Death.	Pulmonary tuberculosis.		Autopsy.
Erythema.	No.	6 mos.	Death.	Broncho-pneumonia.	Broncho-pneumonia.	Autopsy.
Erysipelatous eruption.	No.	2 mos.	Death.	Pulmonary tuberculosis.	Broncho-pneumonia.	Autopsy by Huber.
Urticaria.	No.	5 wks.	Death.	Broncho-pneumonia.	Broncho-pneumonia.	Autopsy by Marchand.
Roseola.	No.	11 wks.	Death.	Broncho-pneumonia.	Broncho-pneumonia.	Autopsy by von Recklinghausen.
Erysipelatous eruption.	Yes.	2½ yrs.	Death.	Acute cirrhosis of liver. Bronc'p'a.	Broncho-pneumonia.	} No autopsy. Muscle } excised during life.
Roseola. Skin pigmentation.	Yes.	16 mos.	Death.	Bronchitis. Myocarditis.		No autopsy.
Urticaria.	Yes.	6 mos.	Recovery.			
Erysipelatous eruption.	No.	6 wks.	Death.	Bronchitis.		Autopsy by Hauser.
Eruption like erythema nodosum.	No.	15 days.	Death.	Pneumonia. Diabetes.	Pneumonia.	} No autopsy. Muscle } excised at death.
Erysipelatous eruption.	No.	2 mos.	Recovery.	Bronchitis. Nephritis.		
.....	No.	3 mos.	Recovery.	Gonorrhoea.		
Erythema. Urticaria.	No.	8 days.	Death.	Bronchitis.		} No autopsy. Muscle } excised at death.
Roseola purpura.	Yes.	2½ mos.	Recovery.	Menorrhagia. Bronchitis.		
Erythema, eczema.	No.	9 mos.	Death.	Broncho-pneumonia.	Broncho-pneumonia.	Autopsy.
Roseola, urticaria.	No.	7 days.	Death.	Bronchitis.	Suffocation.	} Autopsy. Muscle } excised during life.
Erythema.	No.	7 mos.	Death.			Muscle excised during life.
None.	No.	9 mos.	Recovery.			
Erythema. Skin pigmentation.	No.	5 mos.	Death.	Broncho-pneumonia. Round duodenal ulcers.	Broncho-pneumonia.	Autopsy by Wyssokowicz.
Papular erythema.	No.	5 mos.	Death.		Suffocation.	Autopsy.
Erythema. Skin pigmentation.	Yes.	8 mos.	Recovery.			
Erythema.	Yes.	?	?			
Papular eruption.	Yes.	3 mos.	Death.	Epistaxis. Ulcer'n of mucous mem'e.		} No autopsy. Muscle } excised during life.
Roseola. Desquamation.	Yes.	3 mos.	Recovery.	Ulceration of mucous membrane.		
Erythema.	Yes.	4 mos.	Death.	Myocarditis. Ulc'n of mucous mem'e.		No autopsy.
.....	No.	5 wks.	Recovery.	Epididymitis. Tuberc's of knee sev'l yr's prev's to at'k.		
Pustular eruption, erythema, urticaria. Skin pigmentation. Desquamation.	Yes.	2½ mos.	Recovery.			
.....	No.		Recovery.			Muscle excised during life.

RÉSUMÉ OF PATHOLOGICAL FINDINGS.

E. Wagner.—Muscles pale, greyish-red in color. On section the muscles were quite fragile and showed occasionally haemorrhages between the fibers.

Different grades of waxy degeneration to complete destruction of the contractile substance. An extreme grade of fatty metamorphosis. At times both of these changes are seen in the same fibrillae. No vacuoles, but an increase in muscle nuclei. Connective tissue unchanged. Numerous diffuse, very small foci of pus corpuscles. The nerves showed a waxy and fatty degeneration. Muscles of breast, abdomen, neck, upper arm and forearm examined and found implicated.

Potain.—Subcutaneous tissues infiltrated with serum.

Muscles pale and permeated with serum.

Many fibers exhibited granular degeneration. The striations were frequently indistinct. Spleen enlarged. Muscle from left forearm examined and found implicated.

E. Wagner.—Subcutaneous tissues infiltrated with serum.

The muscles mostly of a uniform pale red color. In the flexors of the forearm spots and streaks of very pale appearance alternate with darker areas. The muscles are permeated with serum of a peculiar hard consistency, and are more fragile than normal.

Fibers swollen and showed parenchymatous or waxy degeneration in different stages, as well as fatty changes. The striae faint or invisible. Atrophy of single fibers with simultaneous nuclear increase, or vacuoles without nuclear increase or atrophy. Two or more pathological processes mostly found near each other. Diffuse and circumscribed serous infiltration between the muscles and in the connective tissue. Connective tissue showed proliferated nuclei and single multinucleated giant cells. Regenerative processes of the muscle and connective tissue seen. In places dilatation of capillaries and small haemorrhagic extravasations. Pectoralis major, intercostals, rectus abdominus, diaphragm, triceps brachii, iliacus, gluteus major and soleus examined and found implicated.

Unverricht and Marchand.—Firm, tense oedema of subcutaneous tissues, which are permeated by a yellowish serous fluid.

The muscles are generally of a dark reddish-brown color. They are soft, fragile and without lustre. On cross section pale, slightly transparent, light grey streaks alternate with dark red areas. When one predominates it gives its color to the muscles.

Fibers swollen and showed different stages of a hyaline or waxy degeneration and a finely granular cloudiness. Occasionally fatty degeneration was met with. Longitudinal and cross cleavage. Striae in places completely gone. No vacuoles and no increase in muscle nuclei. Interstitial tissue infiltrated with small round cells. Blood-vessels dilated and filled with blood. Occasionally large or small haemorrhagic extravasations noted. Spleen not enlarged. Extensors of arms (except triceps), extensors of thigh (except sartorious rectus and vasti) and extensors of toes much implicated. Sterno-

cleido-mastoid, pectoralis major and psoas muscles considerably changed. Flexors of thighs and legs as well as muscles of the back only slightly implicated. Abdominal muscles almost unchanged.

Hepp and Jackson.—Firm, tense oedema of subcutaneous tissues. Muscles pale and generally yellow in color, though occasional haemorrhagic areas are seen. They resemble the muscles of a dog and are markedly infiltrated with serum.

Different grades of hyaline or waxy degeneration to complete destruction of contractile substance. No fatty or granular degeneration observed. Striae faint or lacking. No vacuoles and no increase in muscle nuclei. Interstitial changes slight, but round-cell infiltration was found in the perivascular connective tissue or near degenerated fibers; in the latter places especially in the empty sarcolemma sheaths. Spleen enlarged. Rectus abdominis, triceps brachii, quadriceps femoris, pharynx and other muscles examined and found implicated.

Jacoby.—Coarsely granular and waxy degenerations in different stages. Fatty degeneration also noted. Longitudinal cleavage. Transverse striae generally lacking. Vacuoles and an increase in muscle nuclei. Increase in connective tissue, which is myxomatous or fibrous in structure. Perimysium externum, at times, exhibited fatty or waxy changes. Some nerves showed inflammatory changes, or an increase of nuclei in the sheath of Schwann. Left supinator longus and right gastrocnemius examined and found implicated.

Strümpell.—Muscles paler than normal and yellower in color.

Fibers swollen or atrophied and showed a hyaline or waxy degeneration or a finely granular cloudiness. No fatty degeneration noted. Longitudinal and cross cleavage. Transverse striae generally absent. Vacuoles and an increase in muscle nuclei. Occasionally a new formation of connective tissue between the fibers. Typical interstitial foci of small round cells, especially in the perivascular connective tissue. Capillaries dilated and filled with blood. Spleen enlarged. Eye, tongue, pharynx, deltoid muscles of arms, lower extremities and diaphragm examined and found implicated.

Senator.—Subcutaneous tissues infiltrated with a sero-purulent fluid.

Muscles paler than normal.

No muscle degeneration. No vacuoles but an increase in muscle nuclei. Connective tissue increased and oedematous. Interstitial changes most marked. Infiltration of round cells (mononuclear and polymorphonuclear leucocytes) seen everywhere, especially in the perivascular connective tissue. Blood-vessels dilated and filled with blood. Small masses of extravasated blood occasionally noted. Great increase in muscle spindles. Right biceps brachii examined and found implicated.

Fuckel.—Subcutaneous tissues infiltrated with serum.

Muscles somewhat swollen, darker than normal and haemorrhagic. They are quite fragile and lack their normal lustre.

Fibers changed in places to cloudy granules.
Transverse striations partly destroyed.
Muscle from extensors of upper arms examined and found implicated.

Köster.—The muscles were pale, not tense, but quite firm.

Some fibers showed finely or coarsely granular, or hyaline degeneration. No fatty change observed. Longitudinal cleavage. Transverse and longitudinal striae singly or both wanting at times. No vacuoles and no increase in muscle nuclei. Interstitial foci of small round cells especially in the perivascular connective tissue. Capillaries and smaller blood-vessels dilated and filled with blood. Some free red blood-corpuscles in the muscles. Spleen not enlarged. Biceps, pectorals, intercostals, peroneus, tongue, diaphragm, larynx and pharynx muscles examined and found implicated. Pharynx and intercostal muscles most changed.

Kell.—Many fibers swollen and some showed granular and hyaline degeneration. Striae partly or wholly absent. No vacuoles. Between the fibers many large cells with large nuclei (proliferating cells [?]). In places periarteritis.

Spleen enlarged. Supinator longus, pronator, intercostals, heart and gastrocnemius examined and found implicated.

Frohmann.—Granular degeneration of many fibers and loss of cross striations. Waxy degeneration. Increase in nuclei. Peculiar puckering and folding of the sarcolemma. Slight interstitial changes of an inflammatory nature.

Janowsky and Wyssokowicz.—The muscles are pale and of a yellowish-grey color. They are permeated with serum and of a peculiar hard consistency.

Fibers atrophied and showed a granular and waxy degeneration. Marked increase in connective tissue. There was only a slight cellular infiltration. The nerves showed no inflammatory change. Spleen not enlarged. Muscles of back, upper and lower extremities, throat, diaphragm and sphincter ani examined and found implicated.

Bacialli.—Muscles paler than normal.

Fibers swollen or atrophied. They showed different stages of hyaline and waxy degeneration with complete destruction of contractile substance. Longitudinal cleavage. Vacuoles and an increase in muscle nuclei. Marked increase in interstitial connective tissue with abundant infiltration of white blood-corpuscles and here and there small haemorrhagic extravasations. Spleen enlarged.

Oppenheim.—Piece of muscle excised from the gastrocnemius showed the characteristic changes of a severe interstitial and parenchymatous myositis.

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DESCRIPTION OF PLATES.

All of the illustrations are photo-micrographs. The objectives and oculars were the Zeiss apochromatic; the condenser was Bauch and Lomb's, with a numerical aperture of 1; the illumination was the electric arc; Zetnow's light filter with methylene blue was used. The plates were Cramer's isochromatic (medium) and the exposure was two and a half seconds.

PLATE XXX.

Fig. 1. × 656. Objective 6 mm. N. A. 95. Compensat. proj. ocular No. 6. Stain, haematoxylin-eosin.

The muscle anomaly is here well shown in cross section, in the central part of the field. The innermost fibrillae are cut transversely and are surrounded by a longitudinal band of fibrillae, which is somewhat shrunken away from the sarcolemma. The cross striation of the longitudinal band is quite prominent. The anomaly is about equal in size to the other fibers.

Fig. 2. × 570 and reduced. Objective 6 mm. N. A. 95. Compensat. proj. ocular No. 6. Stain, haematoxylin-eosin.

The muscle anomaly represented cut longitudinally. The innermost fiber is contracted and somewhat curled on itself. Its striations are very prominent. To the right are waxy fibrillae, showing longitudinal cleavage and loss of some of the cross striations. The vesicular degeneration of the sarcolemma is to be noted in places.

Plate XXXI.

Fig. 3. × 570. Objective 6 mm. N. A. 95. Compensat. proj. ocular No. 6. Stain, haematoxylin-eosin.

Six of these muscle anomalies, about equal in size to the normal fibers, are seen. Some of the fibrillary bundles are stained deeply and have a homogeneous appearance. The larger one in the lower row shows this waxy change especially well and the innermost border of the longitudinal muscle layer is beginning to be similarly affected. The upper right anomaly shows three fibrillary bundles cut in cross section and surrounded by longitudinal fibrillae. In some the muscle is considerably shrunken away from the sarcolemma.

Fig 4. × 570 and reduced ½. Objective 6 mm. N. A. 95. Compensat. proj. ocular No. 6. Stain, haematoxylin-eosin.

The blebbing or vesicular degeneration of the sarcolemma. In a few blebs nuclei are to be seen. There is a great increase in nuclei in the muscle fiber on the left.

Fig. 5. × 570 and reduced ½. Objective 6 mm. N. A. 95. Compensat. proj. ocular No. 6. Stain, haematoxylin-eosin.

The swollen condition of a muscle fiber filled with vacuoles. The vacuoles are sharply defined, when well focused, and are generally fairly well filled with rather fine granules. A nucleus is to be seen in the innermost vacuole.

Fig. 6. × 570 and reduced ½. Objective 6 mm. N. A. 95. Compensat. proj. ocular No. 6. Stain, haematoxylin-eosin.

The swollen and degenerated sarcolemma sheath is here represented as quite well filled with rather fine granules. The fiber on the right is swollen and shows waxy degeneration together with this granular condition.

Plate XXXII.

Fig. 7. × 570 and reduced. Objective 6 mm. N. A. 95. Compensat. proj. ocular No. 6. Stain, haematoxylin-eosin.

The bursting of the sarcolemma sheath and the spreading out of the fibrillae. Above and below this spot the muscle fiber is contracted. An increase in muscle nuclei is seen. This bursting of the sarcolemma sheath may be due to its weakening caused by the vesicular degeneration at this place, or it may be an artificial phenomenon.

Fig. 8. × 570 and reduced. Objective 6 mm. N. A. 95. Compensat. proj. ocular No. 6. Stain, haematoxylin-eosin.

Increase of connective tissue at expense of the muscle which in places is broken up into bands or shows a waxy condition of the fibrillae. Cross and longitudinal cleavage is here seen. The connective tissue is loose and very oedematous. There is a marked increase in muscle nuclei.

Fig. 9. × 156 and reduced ½. Objective 16 mm. N. A. 30. Compensat. proj. ocular No. 6. Stain, van Gieson.

Increase of the perimysium externum. Many fat cells and new blood-vessels. In the upper part of the field a sarcolemma sheath containing myoblasts is to be noted. The muscle fibers show an increased number of nuclei.

PLATE XXXIII.

Fig. 10. × 570. Objective 6 mm. N. A. 95. Compensat. proj. ocular No 6. Stain, haematoxylin-eosin.

Rod-like nuclei in a row of four are to be seen in the center of a muscle fiber.

Fig. 11. × 570. Objective 6 mm. N. A. 95. Compensat. proj. ocular No. 6. Stain, haematoxylin-eosin.

By direct division of these rod-like nuclei long rows of nuclei can be found. In this figure a line of eleven can be seen.

Fig. 12. × 156. Objective 16 mm. N. A. 30. Compensat. proj. ocular No. 6. Stain, haematoxylin-eosin.

These muscle nuclei are here situated in the hollow of a muscle fiber and in an empty sarcolemma sheath and are surrounded by protoplasm. These cells are known as myoblasts or sarcoblasts.

Fig. 13. × 1390. Objective 2 mm. N. A. 1.42. Compensat. proj. ocular No. 6. Stain, haematoxylin-eosin.

One of the myoblasts seen in the last figure is shown under higher amplification undergoing karyokinesis.

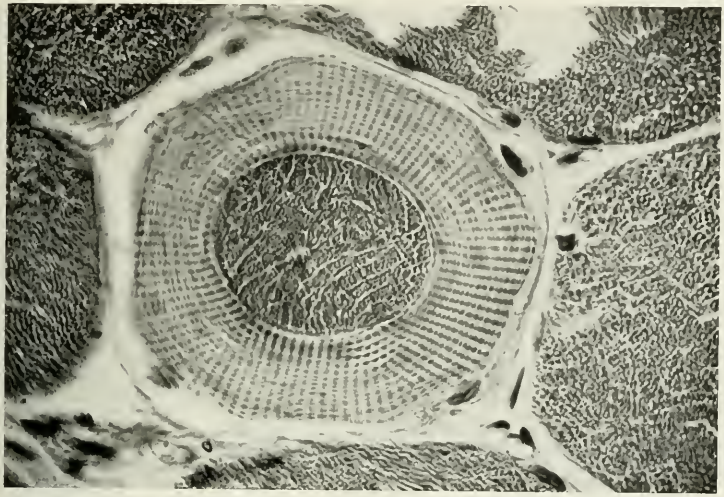


FIG. 1.

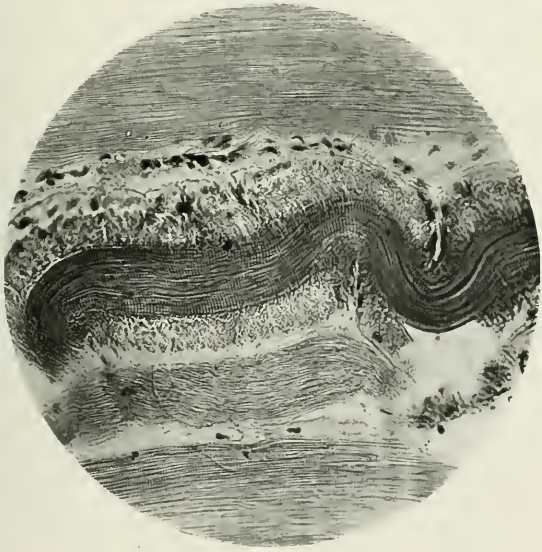


FIG. 2.

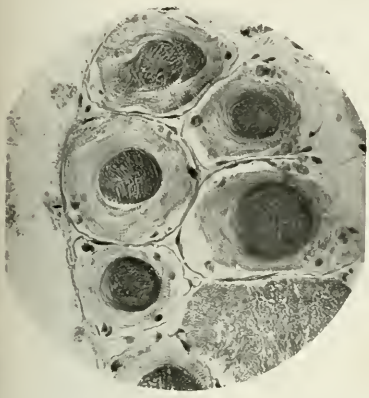


FIG. 3.

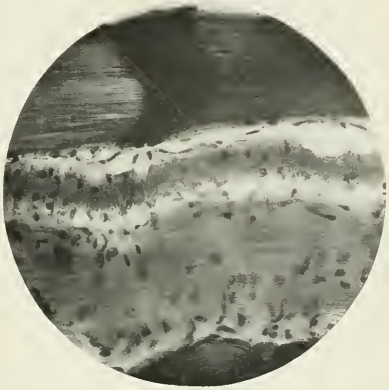


FIG. 4.



FIG. 5.

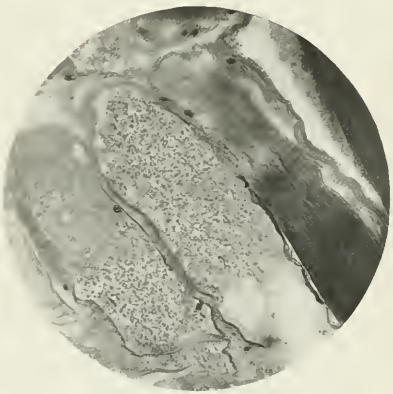


FIG. 6.

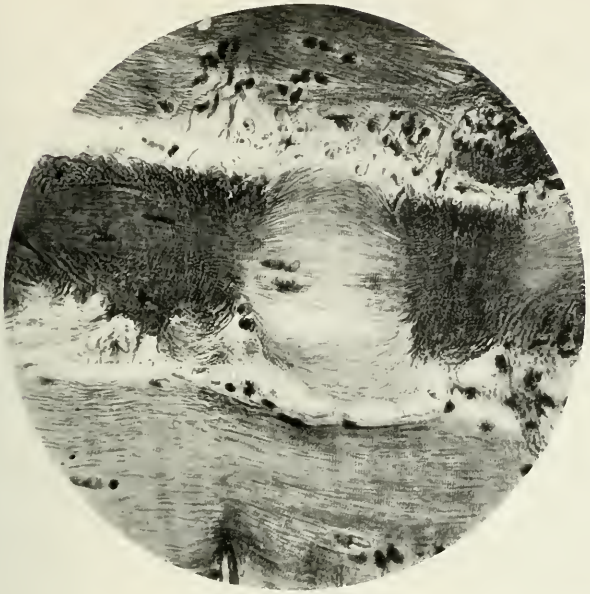


FIG. 7.

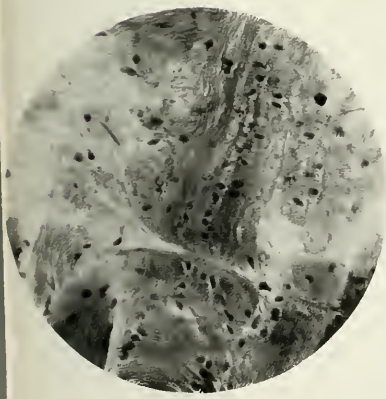


FIG. 8.

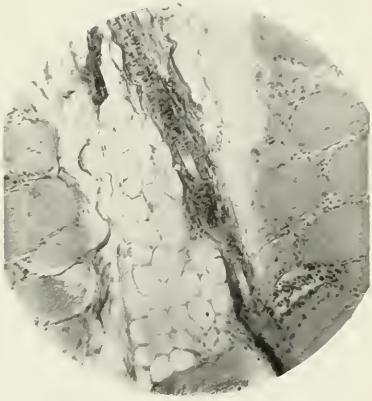


FIG. 9.

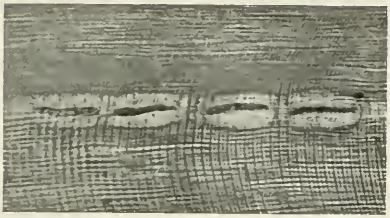


FIG. 10.

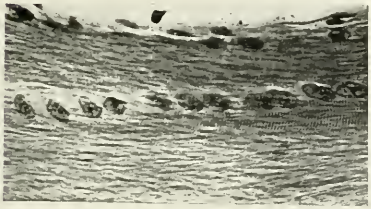


FIG. 11.



FIG. 12.

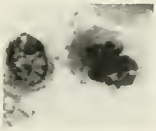


FIG. 13.

FURTHER OBSERVATIONS ON A PATHOGENIC MOULD FORMERLY DESCRIBED AS A PROTOZOON (COCCIDIOIDES IMMITIS, COCCIDIOIDES PYOGENES).

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In June, 1900, Dr. H. C. Moffitt and myself published, in the *Philadelphia Medical Journal,* a preliminary report on "A New Pathogenic Mould," which formerly had been described as a protozoon, and had been named by Rixford and Gilchrist, *Coccidioides immitis* and *Coccidioides pyogenes.* It is the object of this paper to give a more detailed description of the observations communicated in the former article, and also to add some further developments which largely confirm the conclusions which we then drew from our first investigations.

I have also had the good fortune to find another case of this form of infection in a human being, a case that derives an additional interest from the fact that the localizations of the disease in it were in many ways different from those observed in former cases of this kind. Unfortunately the post-mortem examination in this last case was held very late after death and the usual routine bacteriological examination was, therefore, omitted. The unusual nature of the case was not apparent at autopsy and was recognized only when fresh material for animal experiments could no longer be obtained. The appearance of the parasites in the tissues is so typical, however, that a doubt whether this case belongs to the same category seems to be hardly justified.

Apart from a few early reports of coccidiosis in the human being (Hadden, Silcock, Bland-Sutton, all mentioned in *The Lancet,* 1889, II, and in the *Centralblatt für allgemeine Pathologie und pathologische Anatomie,* 1894), of which I have not been able to find descriptions accurate enough to decide whether they belong to our class of cases, there are, so far as I could make out, only four observations of

infections of this kind in the human being recorded in the literature. The first case was found by Posadas in Buenos Ayres, and briefly described by Wernicke in 1892, in the *Centralblatt für Bacteriologie und Parasitenkunde*. With his description of the case he gives some microphotographs which do not leave any doubt about the nature of the infection. Later, after the death of the patient, Posadas published a more elaborate paper on this same case in the *Revue de chirurgie* for March, 1900. The original unfortunately was not accessible to me and I had to be satisfied with a review in the *British Medical Journal* for April, 1900. The other three cases were observed in California, two of them by Dr. Rixford (later described by him in conjunction with Dr. Gilchrist in the *Johns Hopkins Hospital Reports,* I) and the third one by Dr. D. W. Montgomery (*British Journal of Derm.,* XII, 1900). In addition to these and the two cases described in this paper, I know of one more instance that was also seen by Dr. D. W. Montgomery * and examined by Dr. A. E. Taylor. Unfortunately the patient left San Francisco before I could avail myself of their invitation to examine him, and at the same time make some inoculation experiments. It seems, therefore, that the disease is not such a very rare one in California, and we can hope that new material will be forthcoming that will enable us to obtain more positive data about the one important point that now remains to be decided, namely, the source of spontaneous infection. It has remained doubtful whether the disease is transmitted from man to man, or whether the parasite lives somewhere in nature, or, what I suspect very strongly, whether the disease is originally an animal disease and only occasionally transmitted to man.

All former cases, with the exception of Montgomery's, seem to be examples of primary infection of the skin. Rixford and Gilchrist directly speak of a protozoic dermatitis. It is quite remarkable that both cases that I have seen should be different from this usual type. In both of them there were no primary cutaneous lesions

* This paper was written in 1901. Since then Drs. Montgomery, Ryffkogel and Morrow have published this case in the *Journal of Cutaneous Diseases*, January, 1905.

at all, and the infection had evidently started in the lungs and thence spread to other organs. In the first case,[1] the one studied by Dr. Moffitt and myself, the first clinical symptoms appeared in the chest, and at autopsy a very extensive involvement of the lungs was found. There was also an empyema on the left side in which the parasitic organisms and pseudo-diphtheria bacilli were present. From the lungs the disease had extended through adhesions into the diaphragm and further down to the retroperitoneal lymphglands. There had also occurred a generalization of the disease by way of the blood current leading to the formation of miliary abscesses in the spleen, submiliary tubercles in the liver, tubercles and chronic miliary abscesses in both kidneys, purulent periostitis and ostitis of the frontal bone and both tibias, with formation of abscesses and perforation through the skin, suppurative inflammation of both knee-joints, the right shoulder, both elbows and both wrists.

In the second case there were old lesions in the lungs. From the lungs the pericardium had become infected, an infection which resulted in an obliteration of the pericardial sac by fibrous adhesions and the formation of granulation tissue with submiliary tubercles and miliary abscesses in the pericardium at the basis of the heart. Either in the lungs or the pericardium—this point could not be definitely decided—the parasites must have gained access to the blood current, the infection of the blood resulting in this case in a basilar tubercular meningitis and a miliary tuberculosis of the spleen and both kidneys.

These observations prove the occurrence of a primary pulmonary infection in this disease very probably due to inhalation of the parasitic organism which causes it. As yet it is impossible to decide whether a primary intestinal infection by ingestion of the parasite is also possible.

In all six cases the pronounced tendency of the disease not only to produce very extensive lesions at the point of infection but also to spread by the way of the lymph- and blood-current is very marked.

[1] See detailed description of cases at the end of the paper.

All cases ended with the death of the patient. Both facts prove that the human being when once infected is eminently susceptible to the disease, and the scarcity of cases can be explained only by assuming that an occasion for infection is very rarely given.

MORPHOLOGY OF THE PARASITES IN THE TISSUES.

As far as the appearance of the parasites in the tissues is concerned, I have very little to add to the excellent description and very instructive plates given by Rixford and Gilchrist in their paper in the *Johns Hopkins Hospital Reports.*

The adult parasites are almost perfectly spherical organisms measuring about 30 μ in diameter. They consist of a central granular protoplasmic body and a peripheral double-contoured, highly refractile membrane. The thickness of the membrane varies a little, but in normal individuals it always has a considerable diameter—from about a fifth to a tenth of the radius of the whole parasite. Whether there is a definite nucleus has not as yet been made out. The protoplasm stains well with nuclear dyes and there are frequently larger, intensely staining spots in it. Vacuoles may be seen, but are not constant nor even very frequent.[2]

In old lesions one frequently encounters what seem to be degenerated forms. Either they show numerous small vacuoles, or the protoplasm shrinks, and the natural result is the falling together and folding in of the membrane. Such a form is represented in the lower half of Fig. 17. As I have found such forms not only in sections but also sometimes in fresh teased specimens, I do not believe that they can be regarded as artefacts, a possibility that is discussed by Rixford and Gilchrist.

Under favorable conditions the adult forms begin to sporulate. The first step is a cleavage of the protoplasm inside of the capsule into two parts.[3] These divide into four and so on, until a large number of spores have been formed, as is well described in Rixford and Gilchrist's paper and shown in their Figs. 8, 9, 10, 11 and 12

[2] In Rixford and Gilchrist's Case II they were almost constant.

[3] This earliest step, however, has never been actually observed.

in Plate XXVIII. The spores formed in this way are quite numerous, usually a hundred and more. Their protoplasm is homogeneous and highly refractile. The ultimate form of the spores seems to be largely the effect of their being crowded together very closely in the more or less unyielding capsule. The central ones are usually more or less S-like and sickel-shaped. The peripheral ones have the form of biconcave disks or biconcave plates of varying forms. One also finds pyramidal forms with more or less concave surfaces. (Examples of sporulating forms are found in Figs. 5, 16 and 23.)

Neither Rixford and Gilchrist nor I have ever been able to see any active motility in these spores. They sometimes stain very well with nuclear dyes, at other times very badly or not at all. I have the impression that the very last stages are the ones that stain so poorly. At least this much is sure, that after they have been discharged into the tissues through a tear in the capsule—a step that now follows—it is very difficult or impossible to recognize them, because they do not seem to take either nuclear or protoplasmic stains in a decided manner. In a few instances Rixford and Gilchrist have succeeded in staining these free spores with Gram's method.

On account of this difficulty of recognizing the spores after they have been discharged from the capsule, it is impossible to make any very positive statements as to how the young parasites develop from the spores. It is, however, likely that they originate from them by simple enlargement, in such a way that the protoplasm in the center of the spores enlarges and the spore membrane also grows and develops into the double-contoured membrane which envelops the adult parasite. At first there seems to be a considerable stretching of the membrane. At least the younger forms certainly have a thinner membrane than the older ones.

Sometimes, at least in my first case, the development of the young forms from the spores takes place within the old membrane. This seems to occur when the development of the parasite is an especially rapid one. The young forms certainly develop into the adult forms by simple enlargement. One can easily trace the different stages of development in the specimens.

In their Fig. 17 on Plate XXIX, and Fig. 50 on Plate XXXIII, Rixford and Gilchrist depict forms in which the outer surface of the membrane is full of small prickles. The former is a sporulating, the latter an adult form. They state that they observed these forms only in two or three instances out of many hundreds. I have never seen forms with prickles as long as those represented in the plates, which really would be better described as bristles. Short irregularities on the outside of the membrane and numerous short prickles in the same place, however, I have met with very frequently. They are best seen in fresh specimens and so far as my observations go are almost constant on the outside of the membranes of the sporulating forms.

Like Rixford and Gilchrist, I also have been unable to find any definite budding forms. The examples nearest to it, that I have ever seen, are represented in Fig. 29 and Fig. 30. In the latter it certainly looks more as if there was a formation of a small pedicle than that of a bud.

I have, however, quite frequently, especially in my animal experiments, seen two young parasites lying directly adjoining one another so that both together formed a figure 8. I call these, for the sake of brevity, figure-8 forms. In this case each of the two parasites is sometimes surrounded by a distinct membrane, as is shown in Rixford and Gilchrist's Fig. 34 on plate XXX, but at other times the two capsules are fused into one at the point where the parasites touch one another, so that forms are produced which in some cases might easily arouse the suspicion of a budding. However, the two parasites were always of the same size and there was never any direct continuity of the protoplasm.

Rixford and Gilchrist are undecided whether in their two cases they have to do with one and the same organism or whether the organisms in the two cases, although closely related, belong to different species. They say after the description of the second form:[1] "It is not even certain that the parasites in the two cases are not

[1] Rixford and Gilchrist, loc. cit. page 260.

identical species. Still the formation of the spores peripherally and the abundant production of pus with the second form of parasite are such striking peculiarities that for the present we propose the name Coccidioides pyogenes (in contradistinction to Coccidioides immitis) for this second variety of the parasite."

Both in my human cases and also in my animal experiments, which were all made with the parasites obtained from Case I, I have seen so many striking differences in the number and arrangement of the spores formed that I hardly think this point can be used as a means of differentiation between closely related species. As far as the abundant formation of pus in their Case II is concerned, I hope to be able to prove further on that this feature was due not to a difference in the parasites, but to the presence of large numbers of sporulating forms. I believe, therefore, that it will be better, until further evidence to the contrary is forthcoming, to regard the two cases of Rixford and Gilchrist as infections with the same organism.

Rixford and Gilchrist have already shown that with material containing the protozoon-like bodies just described it is possible to produce lesions in animals which, at least to a certain extent, resemble those which were observed in the human being. Subcutaneous inoculations only were attempted and the results of these were not at all constant. Of seven (two dogs and five rabbits) experiments only two (Dog 1 and Rabbit 5) proved successful. In the dog a chronic ulcer developed at the site of inoculation (leg) and an enlargement and abscess formation in the regional (inguinal) lymph-glands followed. In both lesions protozoon-like bodies were demonstrated. Both healed completely after excision. In the rabbit also a chronic ulcer developed which contained the protozoon-like bodies. The ulcer persisted for a long time (six months), but showed a tendency to decrease in size. A part of it was excised, after which the wound healed rapidly. There were no signs of a generalization of the infection.

The material used in these experiments, however, did not contain the parasite in pure culture.

Our experiment on Guinea-pig No. 1 [5] shows that these animals also are not very susceptible to subcutaneous infection. When the guinea-pig was killed ten weeks after inoculation a very slight lesion was found at the point of inoculation and no involvement of the regionary lymph-glands nor any general infection.

That in man also the skin is not very susceptible to the infection Rixford and Gilchrist demonstrated by experimental inoculations of unaffected parts of the skin in their Case I. These inoculations in all instances proved unsuccessful. Our experiments on Guinea-pigs 2 and 3 prove, however, that the animal is very much more susceptible to intraperitoneal infection.

Further experiments with the protozoon-like bodies themselves on rabbits were not performed.

DEVELOPMENT OF THE PARASITE ON ARTIFICIAL MEDIA.

The first to obtain a development of the parasitic organisms on artificial media were Dr. H. C. Moffitt and Dr. May Ash. At the autopsy, which was performed by Dr. Moffitt, they inoculated slanting agar-agar tubes with material obtained from the kidney, lung, the abscess over the left eye, the left pleural cavity and spleen. In the cultures from the pleura and spleen a white mouldy growth developed which, when it was shown to me, I regarded as an accidental contamination, whereas Dr. Moffitt and Dr. Ash were quite ready to believe that it had something to do with the parasitic organism found in the tissues. When, however, Guinea-pig No. 2, which had received some of the pulmonary tissue of Case I injected into the peritoneal cavity, died showing lesions which in many respects resembled those of Case I, and which contained the typical parasites in pure culture, and when also cultures made at the autopsy from the pus near the testicles now developed the same mould in profusion and in pure culture, my attitude towards the mould naturally began to change.

It was at once recognized that in order to make sure of the true relation of the parasite found in the tissues and the mould in the

[5] A detailed record of the animal experiments is given at the end of the paper.

cultures to one another two things were necessary: first, if possible, to trace under the microscope the development of the mould from the protozoon-like bodies (the parasite in the tissues), and secondly, to attempt to reproduce lesions containing these protozoon-like bodies by inoculating animals with pure cultures of the mould. As we have already stated in our preliminary report, we succeeded in both respects.

On April 20, 1900, a little of the pus from an abscess near the testicle in Guinea-pig 3 was suspended in beef-tea in the form of a hanging drop. On examination the drop contained three protozoon-like forms. The slide was then incubated for twenty-four hours and next day a mycelium had developed from two of the three organisms that were present, very much in the same fashion as is represented in Figs. 32 and 33, which are drawn from later experiments. In order to exclude all possible error, this experiment was repeated a considerable number of times, always with the same result.

The experiments showed further that a development of the protozoon-like bodies into mycelia does not take place so long as the parasites are enclosed even in the smallest shreds of tissue. This explains why one usually obtains cultures only from abscesses, not from the more solid tubercle-like lesions.

In the second place, even when the parasites are free, they do not all grow out into mycelia. It seems that they must have at least reached the adult stage.

The development of the mycelia from the protozoon-like bodies occurs in the following manner: The thick membrane which surrounds the latter becomes very thin in one place and begins to evaginate over a bud from the enclosed protoplasm (see Fig. 32, upper left corner). These buds soon assume the form of coarse more or less cylindrical threads [6] which are either straight or somewhat wavy.

[6] In a case studied only very recently after completion of this paper, the first buds were more or less club-shaped, and later on several rows of club-shaped links developed around the protozoon-like body extending more or less in a radial direction away from it. The appearance was entirely that seen in certain saccharomyces that form hyphae in their cultures. More in the periphery of the growth, however, the hyphae became again cylindrical like those in Figs. 32 and 33.

At first the protoplasm forms one continuous mass in main body and buds, but after a while septa appear at various places in the buds and sometimes a partition also forms between the bud and the main body.

Fig. 34 represents what I believe to be the development of hyphae from a spore of the protozoon-like bodies. The specimen was made from an abscess that had developed at the base of the ear of Rabbit 4, after an injection of material from a pure culture of the mould into the subcutaneous tissue at this spot. The specimen, which was taken on the tenth day after the injection, was carefully examined and none of the spores of the mould, originally introduced with the pure culture, seemed to be left. After twenty-four hours in the incubator a budding of hyphae from small round bodies, like *a* in Fig. 34, was found in very many places. Since the pus contained many sporulating forms and also many empty shells, it must have contained many free endospores from the protozoon-like bodies, which, however, at the first examination were not specially noticed. I have stated above how difficult it is to recognize them after they have become scattered in the tissues. It is, therefore, very probable that these endospores enlarged (*a* in Fig. 34 is much larger than the endospores of the protozoon-like bodies) and then budded out into hyphae.

There is one more reason why these round bodies from which the hyphae developed could not be the originally injected spores from the mould, and that is found in their form. The difference is well seen in comparing Fig. 31, which represents a budding spore from a pure culture on artificial media, with Fig. 34.

In our preliminary report we state: "It also seems as if there occurred (in the hanging drop outside of the tissues) a limited development of the protozoon-like bodies when they are enclosed in shreds of tissue." This statement was based on some observations in which I had counted the number of parasites in shreds of tissue before and after incubation. It appeared then as if their number had slightly increased. Later counts, which perhaps were made more carefully, did not confirm the earlier ones, and I now believe that the discrepancy in the figures was due to the fact that after continued soaking in

beef-tea the smaller forms show better in the tissues, so that they could have been overlooked in the first count but were easily seen and registered in the second one.

The cultures obtained from Case I and from a number of the inoculated animals may be shortly described as follows:

Agar-agar (ordinary agar-agar, glycerin agar-agar, 2% glucose agar-agar). The growth develops slowly at room temperature, quite rapidly at 37° C. (thick membrane in 24 to 48 hours in incubator). On the surface of the medium a white mouldy growth is formed with a slightly wavy outline. The aërial hyphae are never very long, about .5 to 1 mm. Old pure cultures look as if they had been strewn over with just visible, bright, white particles of sand. From the lower surface of the growth numerous hyphae grow into the medium, by which the growth becomes very firmly attached to it. Only with a stout platinum needle is it possible to dig out portions of the growth from the surface. The agar-agar in the neighborhood of the growth soon shows a brown discoloration. The growth itself remains perfectly white, only very late when the medium dries out one observes a slight yellowish-brown discoloration. In the later stages of development, when the medium dries out, the growth retracts more in the middle than in the periphery. The membrane begins to show a slight irregular wrinkling but there is no marked folding.

Microscopic examination in the beginning of the development shows long, septate, branched hyphae (about 2 μ in diameter) which form an intricate meshwork. The hyphae within the medium always remain in this condition. In the aërial hyphae one observes later club-shaped swellings at the end and also the formation of spores. The spores originate at the end of the aërial hyphae within them. Three or four are formed at a time by a contraction of the protoplasm at regular distances with accompanying swellings at these places (Fig. 25). Later the contracted protoplasmic mass is shut off at both ends by a double-contoured membrane. The protoplasm that goes to form the spores is at first very granular, later highly refractile and homogeneous. The formed spores have biconvex sides, as is shown in Fig. 25. The places between the spores are entirely devoid of protoplasm and the membranous cylinder that connects the spores after they have been formed, seems to be very brittle, so that after a while the spores break off and form irregular clusters around the ends of the aërial hyphae. In these clusters the spores at first usually adhere to one another in rows, but later they break apart and become

single. At the ends of the isolated spores one can almost invariably see pieces of the empty intermediate link that existed between them.

From this description one will see that the spores are typical chlamydospores and not oidia, as was stated in our first publication. In later cultures, however, I have found oval double-contoured spores in rows at the end of the aërial hyphae. They varied somewhat in size and the largest ones were usually found at the end, giving the whole a somewhat club-shaped appearance. These oval spores were directly attached to one another without any intermediate empty pieces of mycelium. I have never seen any lateral conidia.

In very old pure cultures the spores lose their characteristic shapes and become round or sausage-shaped. They also lose part of their resplendence. When these chlamydospores are brought into fresh media they bud out into new hyphae (see Fig. 31). The spore membrane always bursts at the side.

Gelatine.—Formation of a similar growth occurs on the surface. The aërial hyphae, however, appear late and are not so plentiful. The gelatine beginning with about the tenth day is slowly liquified. Microscopically the growth resembles that on agar-agar. The formation of spores occurs late, however, and is not plentiful.

Raisin-gelatine gives a similar development.

Beef-tea.—Growth at the bottom of the tube in form of irregular flakes that look like pieces of coarse cotton. The growth is first white, later brownish. Microscopically, it consists of a dense felt of septate hyphae of the same appearance as those in other cultures. Formation of air-hyphae and spores was not observed.

Milk is slowly peptonized (in the course of a month or two) without change in reaction. At last one obtains a clear liquid with a thick white scum on the surface, which may show greenish and lilac spots.

Potato.—Abundant growth, very much like that on agar-agar. Early and profuse production of spores.

Carrot and turnip.—Less abundant development and later and less abundant spore formation.

Cultures develop on all media slowly but very well at room temperature (between 15° and 20° C.).

The mould develops very well on all albuminous media (Loeffler's serum, glycerine (6%) serum, ascitic fluid). Protozoon-like bodies were

not observed in any of the cultures even in albuminous media under aërobic and anaërobic conditions.

It was also tried whether a development of the mould into the protozoon-like bodies would take place in a collodion sac that was allowed to remain in the peritoneal cavity of an animal for some time. When the sac was examined five days after its introduction into the peritoneal cavity it contained mould spores only, the mycelium having disappeared completely, but protozoon-like bodies were not found.

I have been able, however, to trace the development of the protozoon-like bodies from the mould after its introduction into the animal body, in Rabbit No. 4. The animal was inoculated into the ear-vein with a culture that contained many spores, but part of the infectious material entered the subcutaneous tissue near the vein. Two days afterwards already a small abscess had developed at the point of inoculation. A microscopic examination of the pus showed that the inoculated mycelial threads had disappeared, but that as yet it contained the injected mould-spores in large numbers and also that a few of them had developed into short hyphae such as are represented in Fig. 28.

On the following (third) day these hyphae began to break up and disappear and had vanished completely on the following day or the day after. With the third day a development of the mould-spores into the protozoon-like bodies began. This development is shown in Fig. 29. As is represented in the figures, the spores either enlarge as a whole (Fig. 29b) and gradually assume the forms of the protozoon-like bodies, or the development is at first uneven. One side of the spore shows a marked bulging (Fig. 29a), but then also in course of time the bodies assume a spherical form and the remnants of the intermediate link disappear. On the fourth day already some of these newly formed protozoon-like bodies had developed into sporulating forms and on the fifth day the sporulating forms were very numerous. On the seventh day the originally injected mould-spores were very few in number and on the tenth day they had disappeared altogether.

In my specimens I have never been able to see any indication of a direct development of mycelium into protozoon-like body, and I do not believe that it ever occurs. In cases in which animals are apparently inoculated with mycelium only, as in the case of Rabbit 2, and in which nevertheless the protozoon-like bodies develop in the tissues, I believe that there must be first a formation of spores and secondarily a development of protozoon-like bodies from them. It may, however, also be that in this case, in the preliminary examination of the pure culture, a few spores were overlooked, which is really more probable.

Inoculation experiments with the pure culture of the mould on guinea-pigs and rabbits were uniformly successful, with the exception of my first experiment on Guinea-pig 4. The culture which was employed in that instance had been developing only a comparatively short time, and the unsuccessful outcome may be explained by a complete absence of spore-formation in the young culture. Otherwise it is difficult to find any reason for it, since in all other cases the infection took very readily.

Two frogs were inoculated, one into the dorsal lymph-space, the other into the peritoneal cavity; they remained healthy and at autopsy did not show any lesions.

In several cases new pure cultures of the mould were obtained by inoculating suitable media with pus from inoculated animals. In all these cases the *pus,* when examined with the microscope, contained protozoon-like bodies in pure culture, and no mycelia, and the *cultures,* which were obtained, mycelia and chlamydospores and no protozoon-like bodies.

I believe that my experiments prove conclusively that the protozoon-like bodies belong to the life cycle of the pathogenic mould, which was cultivated on the artificial media. When the parasite develops in the *animal body* it appears in the form of protoplasmic spheres surrounded by a thick double-contoured membrane and propagates itself by endogenous sporulation; when it develops on the *artificial culture-media* it develops a mycelium, and at the end of the aërial hyphae of this mycelium chlamydospores are formed, which when brought into new artificial media again reproduce the mycelium, and so on. In case, however, these chlamydospores enter the body of susceptible animals, they develop into protozoon-like bodies with endogenous sporulation. These propositions have been proved in two ways. First: Animals have been inoculated with pure cultures of the mould, which did not contain any protozoon-like bodies, and have developed a disease, in the lesions of which the protozoon-like bodies were found in pure culture and no mycelium. From these diseased spots the mould has then again been obtained by cultivation. Secondly: Development of the different stages into one another has been directly traced under the microscope: formation of a mycelium from the protozoon-like bodies and their spores in the hanging drop; development of the chlamydospores into protozoon-like bodies in the subcutaneous abscess in Rabbit No. 4.

CLASSIFICATION.

In regard to the classification of the new pathogenic mould we encounter great difficulties which it has been impossible for me to overcome in a satisfactory manner. I believe that this part of the inquiry belongs rather to the field of botany. I have therefore consulted with several well-known botanists, but they also were at a loss how to place the new fungus, and all seem to agree that our present system of classification of the lower fungi is very imperfect and far from satisfactory in many ways.

The only organism which has at least some points in common with the one described in this paper, is the one that was found by Gilchrist and Stokes[7] in a case of pseudo-lupus. Gilchrist and Stokes observed that blastomycetes in the tissues also form spherical protoplasmic bodies with a thick capsule which, however, multiply by budding and not by endogenous sporulation. When cultivated on artificial media they first show very peculiar budding forms, that are represented on Plates VI and VII, which accompany the article referred to; later one finds a very intricate meshwork of very thin hyphae in the cultures.

I am much indebted to Dr. Gilchrist for giving me the opportunity of studying this parasitic organism. I can only fully confirm Gilchrist and Stokes' observation as far as the growth on artificial media is concerned. In old pure cultures I have regularly found peculiar bodies that seem to develop at the end of the hyphae. They are represented in Figs. 26 and 27. They are about 10 μ in diameter. They consist in a rather thick sometimes double-contoured membranous sac that is filled with small, round, highly refractile granules. These granules do not give the staining reactions of fat. It is possible that they represent a form of endogenous sporulation, but I have not been able to prove this by demonstrating a development of either a new mycelium or the budding forms, that occur in the tissues.

[7] Gilchrist and Stokes. A case of pseudo-lupus vulgaris caused by a blastomyces. *Journal of Experimental Medicine*, 1898, III, 53.

At the meeting of the Chicago Pathological Society on Monday, April 8, 1901, Dr. H. T. Ricketts read a paper on "Blastomycetic (Oidiomycetic) dermatitis and its organisms." As far as I can gather from a short review of his paper, which appeared in the *Philadelphia Medical Journal* of April 27, 1901, he believes that the organisms found in "blastomycetic dermatitis" and also the one isolated from our Case I, although differing in certain respects, all belong to one group of fungi, the oidia. As far as the fungus that is described in this paper is concerned, he has perhaps been slightly misled by our preliminary report, in which it was stated, that oidia had been found in the cultures, yet as far as his contention goes that all these fungi belong together, I agree with him perfectly. A closer investigation, however, has shown that our first statement of the occurrence of oidia was erroneous. The majority of the spore-like bodies are certainly not oidia, but a modification of them—chlamydospores—the characteristic being that in the hyphae the protoplasm is concentrated at certain points in the form of spores, leaving between the spores segments of the original hyphae, which are empty, as is shown in Fig. 25. Following the general laws of oidia and chlamydospores, the chlamydospores formed in our fungus can develop either into a new vegetative form (development of hyphae from them when brought upon new media, see Fig. 31) or under favorable conditions (in our case in the animal body) into "Fruchtkörper" with endogenous sporulation. Following Brefeld's classification of fungi, if I understand correctly, our fungus on account of this constant and typical endogenous sporulation would belong to the ascomycetes.[8]

On account of the difficulties in the way of an intelligent classifi-

[8]Since the above was written, I have had the opportunity not only to study Dr. Rickett's article in the original, but also to see a number of his cultures, which he was kind enough to show me. I am now more than ever convinced that the organisms which he describes and also the one studied in this paper are all very closely related to one another, and belong to a rather badly defined genus interposed between hyphomycetes and blastomycetes. All oidia would belong to the same group and it would perhaps be well to enlarge the meaning of oidium sufficiently to make it cover the entire group, as Rickett in his paper proposes to do.

cation of the parasite, I believe it to be best to reserve the adoption or coining of a generic name for the future, and use the name which has been proposed by Rixford and Gilchrist as a generic name for what they believed to be a protozoon, as a name for the species. Its name then would be *Oidium coccidioides,* or, if there should be objections to the adoption of coccidioides, *Oidium protozoides.*

I have also had an opportunity of studying the blastomycetes that were isolated by Dyer [2] from a case of blastomycetic dermatitis. This organism is certainly entirely different from either Gilchrist and Stokes' or the one described in this paper, growing in all respects like an ordinary blastomyces and causing alcoholic fermentation in sugar-solutions, whereas in the case of the other two parasites I have never seen any evidence of such fermentative action.

LESIONS PRODUCED IN LOWER ANIMALS AND MAN.

A short synopsis of the cases which up to date have been observed in the human being, will serve best to show their similarity in many respects and also to bring out their individual peculiarities.

I. Cases with Primary Cutaneous Infection.

No. 1.—Case of Posadas and Wernicke.—Middle-aged man. First symptom: nodular growths in skin of face and of left thigh resembling mycosis fungoides. Secondary involvement of regionary lymph-glands. Disease lasted seven years. At postmortem miliary tubercles in internal organs.

No. 2. First case of Dr. Rixford and Dr. Gilchrist. Man 40 years old, from the Azores. First symptom: sores on forehead and back of neck. Character of skin lesions: irregular ulcers with fungating masses of granulation tissue containing tubercles. Slowly spreading over face, and infection of other parts of skin. Nine years after first changes in skin involvement of regionary lymph-glands Remittent fever, which lessened after antiseptic treatment of ulcers (fever due to secondary infection with pyogenic cocci?) destruction of both eyes. Death about ten years after first symptoms.

[2] Dyer. Blastomycetic Dermatitis, etc. *American Journal of Dermatology and Genito-Urin. Dis.*, 1900, *No.* 3.

Lesions found at postmortem: Extensive chronic tuberculosis and ulceration of skin. A few small cavities, chronic miliary tuberculosis, old scars and diffuse consolidation of lungs. Chronic tubercular pleurisy and peritonitis. Tubercles in spleen. Multiple abscesses in mesenteric and peribronchial lymph-glands. Chronic tuberculosis of both adrenals. Chronic tuberculosis and suppuration of both testes and prostate. Suppurative necrotizing osteomyelitis of middle of left tibia and head of metacarpal bone of left index finger.

No. 3. Second case of Rixford and Gilchrist. Man 33 years old, from the Azores. First symptoms: pimples on left side of forehead which developed into chronic ulcers. Character of cutaneous lesion: first infiltration of skin, then flat, rapidly growing irregular ulcers. Soon afterwards infection of other parts of the skin. Early involvement of regionary lymph-glands. Metastatic infection of inguinal lymph-gland by way of the blood current. Night-sweats, remittent fever. Duration of disease, three months. No necropsy.

II. Cases with Primary Pulmonary Infection without Cutaneous Infection.

No. 4. *Case* I. Boy 19 years of age, born in the Azores. First symptom: chill followed by pleurisy; later multiple acute arthritis, osteomyelitis of frontal bone. Cough and expectoration. Slight chills and profuse sweating. Duration of disease, three months.

Lesions found at postmortem: Abscesses and diffuse consolidations of both lungs, empyema on left side, purulent infiltration of diaphragm, multiple abscesses and necrosis in retroperitoneal lymph-glands. Miliary abscesses in liver and kidney. Miliary tubercles in kidneys. Multiple foci of purulent osteomyelitis and arthritis.

No. 5. *Case* II. No clinical history; old man, German. Lesions found at postmortem: Old, partly healed lesions in lungs. Chronic tuberculous pericarditis, miliary tuberculosis of spleen and both kidneys, basilar tuberculous meningitis.

III. Case of Apparent Primary Infection of Lungs with Cutaneous Lesions.

No. 6. Dr. Montgomery's case. Male, 21 years of age. German. First symptom: cough ten months before death. Multiple cutaneous lesions appeared two months before death. Character of cutaneous lesions: Large tubers like those of mykosis fungoides, later suppuration.

High remittent fever with night-sweats. Secondary involvement of regionary glands. Marked rapidly progressing pulmonary lesions.

Findings at postmortem: Cutaneous lesions. Large abscess of the size of an infant's head in upper part of right lung communicating with abscess of the neck and the chest underneath the clavicle on the right side. Several smaller abscesses and diffuse consolidation of right lung. Immense abscess of liver. Kidneys and spleen apparently normal.

A comparison of these cases shows in the first place that the duration of the disease varies within wide limits (from three months to nine years) and also that the duration in no way depends on the primary seat of infection.

The clinical symptoms vary very much and of course depend entirely on the organs affected, and on the extent of the lesions in them. Remittent fever and night-sweats seem to be a constant feature of the disease, at least in its later stages. They were present in all four cases of which I found clinical records.

In all cases the disease shows a marked tendency to spread as well by way of the lymph-channels as by way of the blood-current. Case 2, however, proves that this tendency to generalization may manifest itself very late (nine years after the beginning of the disease).

The cutaneous lesions, when they are present, may apparently be quite different in different cases, showing either more the appearance of mykosis fungoides with large tubers and secondary slow ulceration (Cases 1 and 6) or that of a chronic tuberculosis verrucosa cutis (Case 2); or again one observes rapid infiltration of the skin with extensive flat ulceration (Case 3). The differences are due to the more or less rapid progress of the destructive process.

In all organs the disease appears in the form of miliary nodules or miliary, rarely larger, abscesses, which exceptionally, however, may attain a very considerable size (abscess of size of child's head in lung and immense abscess in liver in Case 6).

The disease, therefore, belongs to the infectious granulomata and shows a great deal of similarity to glanders. In fact, when I first saw the specimens of Case 5, I at once thought of glanders. As a name for the disease I should propose *coccidioidal granuloma.*

What is true in these respects of the human cases, also holds good for the disease in guinea-pigs and rabbits, as a study of the detailed accounts of the animal experiments, which are given at the end of the paper, will readily show.

I should like to call special attention to the frequent multiple infections of the bone (Cases 2 and 4, Rabbit 3 [osteomyelitis of some lumbar vertebrae with compression of the cord, osteomyelitis of the parietal bones with compression of the cerebrum]).

The infection of the testicles and prostate in human Case 2 acquires interest when we observe the constant occurrence of suppurative periorchitis in guinea-pigs after intraperitoneal infection. *En passant* I should like to point out that here again we have a new proof that suppurative periorchitis in guinea-pigs is not pathognomonic for glanders, as is maintained by some authors.

Although frequently the lesions have a marked tendency to progressive development, they are chronic in so far as even the abscesses are always surrounded by a considerable amount of cicatricial tissue. Individual lesions may heal spontaneously, as is well shown in Case 5 and in Rabbit 3 (see also Fig. 21). Sometimes the healed, or nearly healed, nodules show calcification. But while this healing tendency may be well marked in certain parts of the body, the disease itself always seems to progress to a fatal termination. At least such has been the case in all human cases, and in all animals that were not killed prematurely or were not cured by surgical interference (Dr. Rixford's cases).

The *histology* of the lesions is of the greatest interest from a general pathological standpoint. In the first place they furnish proof that the typical tubercle (consisting of epithelioid cells and a peripheral zone containing lymphocytes and plasma cells) with giant cells of the Langhans type and central caseation, is not absolutely characteristic of an infection with tubercle bacilli. Our parasite in the human being as well as in animals can produce lesions which histologically cannot be in any way differentiated from the nodules produced by tubercle bacilli (see especially Fig. 7).

In the second place we observe in this disease remarkable transitional stages between nodular lesions on one hand, and chronic miliary abscesses on the other. There are nodules with central abscess formation (see Fig. 18) and chronic abscesses with central caseation of their contents.

Furthermore, sometimes we find large masses of fibrous tissue with numerous very large fibroblasts, few leucocytes and occasional giant cells usually including one or more of the parasites. These masses in their structure bear a certain resemblance to some tumors of the class of fibrosarcomata or fibromyxosarcomata.

It may appear extraordinary that one parasite should be able to produce lesions of such entirely different aspect. A careful study of the tissues, however, has convinced me that these differences are largely dependent on the stage of development in which the parasite is present. The adult forms are found in the less acute lesions, whereas the abscesses always contain sporulating forms, often in very large numbers. A study of the illustrations given by Rixford and Gilchrist seems to prove that this holds good for their cases also. It is true that occasionally one finds a sporulating form in a nodule, as is represented in Fig. 23, but in all these instances the capsule which surrounds the spores is intact, or seems to have burst only recently. It seems then that only after the bursting of the capsule the substances which produce the suppurative process gain access to the tissues.

The fact that the sporulating forms contain a substance which has a strong positive chemotactic influence upon the polymorphonuclear leucocytes is also evidenced by their very frequently entering into them soon after the capsule has burst, and often collecting in them in large numbers. In our Fig. 16, a polymorphonuclear leucocyte is just entering, as it were, the capsule through a small opening, whereas our Fig. 13 and Fig. 8 in Rixford and Gilchrist's Plate XLI show empty cells full of polymorphonuclear leucocytes. This allows us to understand also why in Case 3 with very rapid course and much suppuration sporulating forms were so plentiful.

That part of the poisonous substances which are elaborated by the parasite are absorbed and cause severe general disturbances in demonstrated by the fever and the night-sweats in the human cases. It even seems that sometimes the absorbed toxins are powerful enough to produce focal necroses in the liver, which are so common in other infectious diseases. At least this is the only interpretation that I can give to the peculiar lesions which were found in the liver of Rabbit 2, and are represented in Fig. 15.

I am doubtful whether the slight diffuse cirrhotic changes in the liver of Rabbit 3 are also due to this general intoxication, or whether they constitute an accidental complication.

CONCLUSIONS.

From the preceding I derive the following conclusions:

1. The disease which formerly has been described as a form of protozoon-infection is due to an infection with a pathogenic fungus.

2. The infection may primarily be either a cutaneous or a pulmonary one.

3. The lesions produced by this fungus fall under the general head of infectious granulomata and consist partly in nodules resembling altogether those produced by the tubercle bacilli and partly in chronic abscesses.

4. The adult forms of the parasite are more apt to produce nodules, the sporulating forms abscesses.

5. The fungus is pathogenic for dogs, rabbits and guinea-pigs probably other animals also, and in them produces lesions very similar to those which we encounter in the human being in this disease.

6. Supurative periorchitis in guinea-pigs is not pathognomonic for glanders.

In concluding I should like to express my obligations to Dr. H. C. Moffitt, who gave me the opportunity to examine Case 1, and who allowed me to make use in this paper of the first animal and culture experiments which he performed together with Dr. May Ash.

Since finishing this paper I have seen two more cases of this peculiar affection. One was the unpublished case of Drs. Montgomery and Taylor, mentioned at the beginning of the paper.[10] It was a very typical one with primary cutaneous lesions resembling chronic hypertrophic lupus. With these lesions the patient suffered for a considerable time before he died of an acute generalized miliary (pseudo-) tuberculosis produced by the dissemination of the fungi by way of the blood-current. This case closely resembled Case 2 of the former series. Through the courtesy of Drs. Taylor and Ryffkogel I was able to investigate the case thoroughly before and after death and confirm my former findings in regard to the nature of the parasite and the character of the lesions. The second case was more unusual. The patient, who was under Dr. Morton's care at the German Hospital in San Francisco, was a Japanese section hand employed by the Santa Fe Railroad. He had a chronic ulcer of the right foot just below the ankle on the inside. It was fairly regular, from 3 cm. to 4 cm. in diameter, and covered with a thick brown scab. The edges were very slightly elevated and reddened, the surrounding skin being perfectly smooth and very nearly normal. There was hardly any discharge. The femoral and inguinal lymph-glands became involved, were removed and sent to me for examination. They looked exactly like chronic tubercular lymph-glands with very extensive dry, diffuse caseation. In sections from these lymph-glands, which otherwise presented all the microscopical changes produced ordinarily by chronic tuberculosis, the parasites were discovered; later they were also demonstrated in the primary lesion. Cultures in hanging drops were made with the usual result. After removal of the original ulcer the patient was discharged in an improved condition but not completely cured. There was one fairly large lymph-gland on the right side of the neck, the removal of which was deemed inadvisable. No history, unfortunately, could be obtained from the patient, who spoke Japanese only.

The last case is particularly interesting on account of the unusual

[10] Now published l. c.

appearance of the primary cutaneous lesion and also on account of the extensive dry diffuse caseation in the extirpated lymph-glands.

DETAILED DESCRIPTION OF HUMAN CASES AND ANIMAL EXPERIMENTS.

Case I.—A. L., aged 19 years; farm laborer; born in the Azores. Entered Ward C, City and County Hospital, January 26, 1900. Died Feb. 6, 1900.

Past History.—Unknown.

Present Illness.—Began eleven weeks ago with a chill. Had been riding in the rain after cattle. Went to bed, and after a few days left pleura was tapped, and one gallon (?) clear fluid obtained. Had pain in left side and back. Three weeks after beginning of illness went to St. Luke's Hospital. There had irregular fever, at times reaching 104.4°; nosebleed. Abdominal tenderness; diazo reaction present; no Widal reaction (two examinations). Leucocytes 7000 in cmm. Movements at first pea-soup-like in character, later solid. Dullness over left apex, later at left base. Tapped January 23, three days before admission to City and County Hospital, with negative result.

Four weeks before entrance (about January 1) joint trouble began. At first there was pain below the left patella along the tendon. One week later left knee-joint swelled. Then right knee, right shoulder, both elbows and wrists swelled, and grew red and tender. There were no remissions of pain or swelling. For three weeks before entrance great pain in frontal region, which it was impossible to relieve.

Profuse sweating. A large gland developed in left supraclavicular fossa, a week before entering City and County Hospital, also a fluctuant swelling over left eye. Cough with muco-purulent and occasionally blood-streaked expectoration. No tubercle bacilli in sputum.

Examination (January 27).—Emaciated, yellowish pallor, flushed cheeks. Limbs helpless, due to swelling of joints and pain. Right wrist swollen, red and tender. Right elbow and shoulder red and painful. Both ankles and feet swollen, tenderness over small bones of foot, marked on the left. Both knees swollen and tender. Pain marked along patellar tendons and over crests of tibiæ; evident periostitis of left tibia, less distinct on right. Over left eye a fluctuating swelling, the size of a walnut, starts from frontal bone and is very tender but not reddened.

Long eyelashes, pupils normal. Tongue tremulous, not much coated. Rales heard in mouth. Small glands in submaxillary fossæ and above right clavicle. Large gland the size of walnut over left clavicle. Below

this, reaching downward to the pleural space, is an abscess the size of a lemon. General icterus.

The lower intercostal spaces on both sides bulge. Dullness above both clavicles, more marked on left side. Dullness on right side below clavicle, reaching outward to axilla. Dullness in right axilla. Lung almost tympanitic anteriorly from 1st to 4th rib. Dullness along the 4th rib; deep liver dullness at 5th interspace. Left lung dull over its entire anterior surface; absolutely flat in axilla. On auscultation loud bronchial breathing at right apex; a few rales. A few mucous rales at base. On left side bronchial breathing at apex. Anteriorly coarse and fine, dry and moist rales. Heart large, no alterations in sounds.

Specific gravity of urine is 1020; no albumin; no sugar; indican present; diazo reaction present.

Plantar reflexes normal.

Jan. 31. Leucocytes 17,000 in cmm.

Feb. 4. Slight chills. Profuse sweating.

Feb. 6. Large mucous rales at the base of the left lung.

Tubercle bacilli were not found in spite of repeated examinations of the sputum. The patient showed progressive wasting and died Feb. 6, 1900.

The *autopsy,* by Dr. Moffitt, showed:

Right lung: Irregular, nodular consolidations with necrotic centers from the size of a shot to 1 cm. in diameter. The tissue between them was in great part atelectatic; in the middle lobe were several abscesses with thick abscess-membrane. Left lung: Acute broncho-pneumonia and chronic interstitial pneumonia with abscess-formation; also nodules like tubercles as on right side. There was a large abscess partly in the mediastinum, partly in the left lung.

The pillars of the diaphragm were infiltrated with pus, and there were necroses and abscesses in the retroperitoneal lymph-glands. The right pleura was free; in the upper part of the left pleura an encapsulated empyema was found; in the lower part adhesions.

The spleen was enlarged and softened; in the pulp were some ill-defined yellow patches.

The kidneys showed cloudy swelling; in both of them were miliary nodules with central caseation or miliary abscesses with thick abscess-membrane. The nodules and abscesses were mostly in pyramids.

In the liver were cloudy swelling and fatty infiltration, but no visible nodules.

There were purulent ostitis and periostitis in the frontal bone over the

left eye and in the upper part of the left tibia, and suppuration in the affected joints. Necroses and abscesses in regionary lymph-glands.

Microscopic Findings.—Lungs (see Figs. 2, 3, 4).—Sections of the lungs are full of irregular, miliary and larger, often conglomerate nodules. Many of them seem to have originated in the perivascular and peribronchial connective tissue, whereas some seem to have formed in the pulmonary tissue proper, but even those which arise in the peribronchial or perivascular connective tissue, have pushed their way into the adjoining pulmonary tissue. The structure of the majority of the nodules is the following (Fig. 4): The air cells in the affected area are filled with a cellular exudate which consists mostly of epithelioid cells and lymphocytes imbedded in a meshwork of fibrin. Many of the lymphocytes show the characteristic appearance of plasma cells. Among the cells there are also a few polymorphonuclear leucocytes. One also finds large giant cells, many of them with a peripheral arrangement of their nuclei. The centers of the nodules often show extensive caseation (not present in figure) with much nuclear debris in a necrotic, finely granular material. There is a little, but not much, fibrin between the cells and in the caseated matter. In a few places a few connective-tissue fibrils can be seen between the epithelioid cells. In other places (Fig. 5) abscess-like collections of polymorphonuclear leucocytes may be seen in the middle of the caseous areas. Many of the polymorphonuclear leucocytes, especially those in the middle of the abscesses, show signs of beginning disintegration (pyknosis, karyorrhexis). In the pulmonary tissue between the nodules the air-cells are partly collapsed, filled with coagulated oedema and large desquamated epithelial cells with some lymphocytes and a very few polymorphonuclear leucocytes. In no place, even in the nodules with more chronic appearances, do the septa participate much in the diseased process. They usually show only a moderate infiltration with lymphocytes and plasma cells. In a few spots the lesions show a more acute character throughout. In these places all the air-cells are filled with polymorphonuclear leucocytes, desquamated epithelium, oedematous exudate or a little fibrin. In the middle of such areas there is usually evidence of beginning degeneration and nuclear fragmentation of the leucocytes.

Parasites are present in large numbers in all stages of development. In the tubercle-like nodules one finds mostly young and adult forms (see Fig. 4), almost invariably enclosed in giant cells. In the abscesses there are a very large number of sporulating forms (see Fig. 2), with very thick membranes, which are often prickly on their outer surface. In most of them the spores have already developed into young forms. In

many places one can see that the polymorphonuclear leucocytes have entered the interior of the capsule in which the spores lie through the opening formed by its bursting.

The tissue is in a bad state of preservation. Everywhere one finds very many large bacilli in chains, resembling morphologically anthrax bacilli. They do not show any constant relation to the lesions in their position, and are most abundant in the blood-vessels so that one seems justified in assuming that they are organisms of putrefaction (they did not develop in any of the cultures). Mycelia were not found in any place.

Kidneys (Figs. 5 and 6).—Very many miliary and submiliary chronic abscesses are present. The contents of the abscess-cavities consist of polymorphonuclear leucocytes, some lymphocytes, and a considerable number of epithelioid cells. Around the abscess cavities there are layers of granulation tissue with many epithelioid cells, more peripherally lymphocytes (mostly plasma cells). Giant cells are present, but are not very numerous. The largest abscesses show central necroses. In these necrotic areas dead polymorphonuclear leucocytes, etc., are fused together into a dense granular substance which resembles very much caseous material, and is full of nuclear debris. In other places (Fig. 6) there are more tubercle-like nodules, which consist largely of epithelioid cells. In the periphery of these nodules one finds many lymphocytes and plasma cells. There may be some giant cells and in spots beginning caseation. Extensive necroses are seen in the epithelial cells of the convoluted tubes and ascending loops of Henle.

Parasites are numerous, and are found in all stages of development. In the middle of the abscesses there are many sporulating forms (Fig. 5) or spore membranes enclosing many young parasites. No other bacteria, no mycelium.

Liver (Fig. 7).—Very small submiliary nodules, which consist largely of epithelioid cells and lymphocytes (many plasma cells) occur. The lymphocytes and plasma cells are mostly in the periphery of the nodules. Among the epithelioid cells one may find a few large giant cells of Langhans' type. The centers of the nodules are often caseated. Moderate numbers of young and adult *parasites* appear, almost invariably in giant cells. In a few of the nodules sporulating forms, surrounded by a small gathering of polymorphonuclear leucocytes, are encountered. No other bacteria, no mycelium.

Spleen (Fig. 8).—Many submiliary abscesses with some epithelioid cells in the periphery are present. In some of them are apparent central

necrosis and formation of granular or fibrinous deposits with much nuclear debris. Many *sporulating forms* are seen in abscesses. In a few spots are tubercle-like nodules consisting of epithelioid cells, lymphocytes, and giant cells, and containing a few young or adult forms. No other bacteria, no mycelium.

Stomach.—Some atrophy of mucous membrane, superficial (post-mortem) necrosis, otherwise normal.

Sections from all organs were stained for tubercle bacilli and carefully searched for them, but with negative result.

Parasites were also found in abundance in the pus from the abscess over the left eye. Here they were associated with staphylococci and streptococci. In the left pleura (empyema) they were also present in large numbers together with pseudo-diphtheria bacilli.

Case II (Service of Dr. Hirschfelder).—G. B., German, admitted May 8, 1900, from County Jail No. 2.

In spite of all endeavors it has not been possible to obtain any information in regard to his former history.

Patient entered hospital in an unconscious condition, breathing stertorously. Pupils small, reacting equally to light. Head turned to right. Slight paresis of muscles of right side of face. Muscles of extremities rigid. No pulmonary dullness. Outline of cardiac dullness normal; tones clear; pulse full, regular. 96. Respirations 40. Sensation evidently not impaired. When forehead is pricked with pin patient slowly raises hand to head. With exception of paresis of right side of face, no paralysis or paresis can be made out. Voids urine and feces involuntarily. Urine removed by catheterization. Acid, no albumin, no sugar, no diazo-reaction, no indican.

May 9. Patient somewhat brighter. Evidently understands what is said. Puts out his tongue slowly when told; closes and opens his eyes when told. Drinks when cup is held to his lips.

May 10. Condition unchanged. No dullness over lungs.

May 11. Condition about the same. Patient still unable to speak.

May 12. Same.

May 13. Same.

May 14. Patient died at 2.40 P. M.

Autopsy Record.—96. G. B. Aged 50 years. Laborer. German. Died May 14, 1900, 2.40 P. M. Autopsy May 16, 1900, 3 P. M.

Moderately strongly built, fairly well nourished man. No enlargement of superficial lymph-glands. Abdomen moderately distended. No

lesion on external genitalia. Diffuse brown discoloration of the skin. Slight cyanosis of both ears. Beginning green discoloration of abdominal wall. A few drops of slightly bloody fluid in recto-vesical pouch. Diaphragm, 5th rib on both sides. Thorax well arched, symmetric. A few drops of slightly bloody fluid in right pleural cavity. Both layers of the pericardium attached to one another by moderately firm adhesions; pericardial space entirely obliterated.

Heart.—Post-mortem clot in pulmonary artery. Valves normal on both sides; slight atheroma of aorta. Cavities of heart normal size. Muscle soft and flabby. At the base of the heart the pericardium is much thickened. In spots the newly formed cicatricial tissue is infiltrated with a little purulent fluid. In other places there are a few, just visible, greyish-white or yellow nodules.

Lungs.—A few stringy adhesions at the apex of the left lung. Pleura over left lung otherwise normal. The lung itself is a little cyanotic. There is a little emphysema along the free margin. A little muco-pus in bronchial tubes. The mucous membrane of the bronchi is slightly reddened. At the apex near the adhesions there is a small black scar. Slight thickening of pleura at right apex. Otherwise right lung same as left.

Spleen.—A little large, 13 x 11 x 4.5 cm. It is soft. The cut surface has a brick-red color and the markings are indefinite.

Both *adrenals* normal.

Left kidney a little large. Surface studded with greyish-white submiliary nodules. The nodules are solid and some of them are surrounded by a hyperæmic zone. A few similar nodules in cortex on cut surface. Pelvis of kidney normal. Right kidney is in the same condition as the left one.

Appendix is of normal caliber and length. It is found curled up in a little peritoneal pouch, which is situated on the median side of coecum, directly under the lower end of the ileum and within the mesentery. This pouch is completely shut off from the rest of the peritoneal cavity by a thin transparent membrane. The *sigmoid flexure* is fastened to the brim of the pelvis by some white stringy adhesions.

A little turbid urine was found in the bladder.

Bladder, prostate, seminal vesicles, rectum, right *testis* normal.

No enlargement of *mesenteric glands.*

Bile-duct is open.

A little dark brown mucus in *stomach.* Extensive post-mortem digestion in fundus. Atrophy of mucous membrane in pyloric region.

Pancreas normal.

Liver very much decomposed and full of gas bubbles. Gall-bladder and contents normal.

Thick white coating on upper surface of *tongue*.

Pharynx filled with muco-pus. *Tonsils* slightly enlarged.

Oesophagus, larynx, trachea, thyroid, thoracic aorta normal.

Some of *peribronchial lymph-glands* are enlarged and show numerous black spots on cut surface.

Longitudinal sinus contains a few post-mortem clots.

Much oedematous fluid and gas in *pia mater*.

Venous sinus at basis of skull normal.

The pia mater at the *basis of the brain* and more especially in the Sylvian fissures is diffusely infiltrated with pus and full of submiliary yellow nodules. Large numbers of similar nodules are found at the bottom of the longitudinal fissure of the brain. A little turbid fluid in both lateral ventricles. On both sides of the septum the ependyma is studded with just visible, translucent, greyish-white nodules. The substance of brain is a little oedematous and anaemic, otherwise apparently normal.

Anatomical Diagnosis.—Small scars in both apices of lungs. Pleuritic adhesions. Purulent infiltration and (pseudo) tuberculosis of pericardium at basis of heart. Chronic pericarditis with complete obliteration of pericardium. (Pseudo) tuberculosis of meninges, spleen and both kidneys. Ependymitis chronica nodosa.

Microscopic Findings.—Brain (basis, Figs. 9 and 10).—Pia mater very much thickened, hyperæmic; it contains many small abscesses. Most of them are more or less spherical, others more irregular in form. At their central portions one finds irregular masses consisting of fibrin, fragments of nuclei, bodies and fragments of small round cells, some intact and some broken-down blood-corpuscles, a few intact cells, most of which are polymorphonuclear leucocytes. Further outwards follows a zone in which there is less fibrin, forming long slender fibers woven into a delicate network. In the meshes are many polymorphonuclear leucocytes and a few lymphocytes. In the periphery of the abscesses one encounters mostly lymphocytes, many plasma cells and large mononuclear cells, which often enclose in their bodies lymphocytes, or one or more of the few polymorphonuclear leucocytes which are also present. Other cells of this kind are very large and have a vacuolated appearance, very much as if they had been loaded with fat. There is no evidence of a formation of connective-tissue fibers between the large mononuclear cells. In some abscesses one finds large giant cells, some of which are of the Langhans type, whereas in other

cells the nuclei are situated in the center. In a few spots the character of the lesion differs from what has just been described. In these places one finds nodules consisting almost entirely of large, more or less irregular, mononuclear cells, with lymphocytes and plasma cells. The center of them is frequently necrotic. These nodules, which resemble very much recent tubercles, sometimes contain giant cells. The tissue between nodules and submiliary abscesses contains much fibrin; in many places there are hæmorrhages. It shows a diffuse irregular infiltration with all the different form of cells mentioned, with the exception of giant cells. The blood-vessels are hyperæmic; the smaller ones show marked marginal disposition of leucocytes. A few smaller ones are obliterated by thrombosis. The thrombi contain many leucocytes of different forms. Smaller, not occluding, thrombi of a similar kind are present in some of the larger blood-vessels.

The *parasites* can be found in the abscesses and nodules, but they are present in small numbers only—apparently one or two in each abscess or nodule. In most cases one finds empty shells; in other instances are found sporulating forms with many oval spores in a thick double-contoured membrane. The spores stain hardly at all with any of the dyes that were used (hæmatoxylin, eosin, methylene blue, Van Gieson's stain, carbolfuchsin). Spore-like bodies may also occasionally be found in the surroundings of an empty burst shell. Quite often these empty shells are filled with leucocytes, mostly polymorphonuclear forms. Only rarely one finds young forms. The parasites are often enclosed in giant cells.

No tubercle bacilli nor any other bacteria are found.

Testicle.—No changes.

Spleen.—Slight brown pigmentation. Pulp rich in large mononuclear cells and plasma cells. In one Malpighian body is a submiliary nodule consisting of epithelioid cells.

Kidney.—The nodules consist of a peripheral layer of connective tissue. Inside of this layer of connective tissue there is a layer of tissue consisting mostly of large irregular mononuclear cells, and also some capillaries. The rest of the cells are lymphocytes and plasma cells. In this layer there are several giant cells mostly of the Langhans type. The center of the nodule is formed by a necrotic mass, in the periphery of which one can recognize the former structures in outline. The center is structureless and finely granular. In parts of the necrotic material one finds irregular fragments of nuclei. Almost all epithelial cells in the convoluted tubules and in descending loops of Henle are necrotic.

Stomach.—Marked atrophy of mucous membrane and necrosis of upper layer.

Pericardium at basis of the heart (Fig. 7).—Marked thickening of both layers by development of fibrous tissue. Between the basis of the aorta and the thickened pericardium there are isolated nodules which resemble very much tubercles. They also occur in small groups. They consist of epithelioid cells and lymphocytes. The lymphocytes are more numerous in the periphery and many of them have the appearance of plasma cells. Among the epithelioid cells there are large giant cells of the Langhans type. Some of the nodules show central caseation, in others a fine reticulum of connective-tissue fibers is visible between the epithelioid cells. In one spot the center of a small nodule is completely disintegrated, and the space produced by the destruction of the tissues is filled in with polymorphonuclear leucocytes and some lymphocytes. Between the nodules and outwards from them there is a very thick layer of dense scar tissue, in which there are a considerable number of "Mastzellen." In the neighborhood of the pulmonary artery the vasa vasorum are surrounded by a cellular tissue consisting largely of lymphocytes and fibroblasts. A small vein which runs through the granulation tissue is partly obstructed by a thrombus which contains many polymorphonuclear leucocytes. No parasitic organisms in thrombus.

Parasites are found in considerable number in nodules at the basis of the aorta, mostly young and adult forms; in places where a healing tendency is marked one often finds empty shells. No sporulating forms were seen. No tubercle bacilli and no other bacteria except clumps of short pole-staining bacilli in some of the blood-vessels. No parasites in the granulation tissue in the adventitia of the pulmonary artery.

Lungs.—A section from a small scar near the apex of one lung contains a thick mass of scar tissue with hyalin degeneration and central necrosis; no parasites; clumps of bacilli in some of the blood-vessels. A section from the lower lobe shows hyperæmia and partial collapse. Bacilli in bronchioli and in some of the blood-vessels.

In a section from a large scar the pulmonary tissue is entirely collapsed; septa somewhat thickened by formation of granulation tissue in them; many giant cells in collapsed air spaces. Large areas of granulation and cicatricial tissue. In granulation tissue nodules of epithelioid cells with giant cells; some show central caseation. In one nodule complete disintegration of center with formation of small cavity which is filled with albuminous detritus and nuclear debris.

Parasites (adult forms and empty shells) in nodules; no sporulation forms; no tubercle bacilli or other bacteria.

Appendix.—Atrophy of mucous membrane. Thickening and induration of submucosa.

Guinea-pig No. 1 (Drs. Moffitt and Ash). Feb. 7, 1900.

Piece of lung inserted under skin. Killed April 20. At the point of inoculation a thick scar had developed. In the scar there were a few just visible yellow spots, and in them a few adult forms of the protozoon-like bodies were found. No lesions in regionary lymph-glands or internal organs.

Guinea-pig No. 2, male (Drs. Moffitt and Ash). February 10, 1900.

Particles of lung of Case I suspended in normal salt-solution injected into peritoneal cavity; about end of second week, the animal shows loss of customary activity. On or about February 26, animal showed a well-marked orchitis and swelling at the site of inoculation, with gradual emaciation. The guinea-pig was killed with chloroform on March 2.

Findings at Autopsy.—Suppuration around both testicles; enlargement and inflammation of inguinal glands; white miliary and submiliary nodules in spleen, diaphragm and lungs. Fresh specimens from the different organs showed the protozoon-like bodies in different stages of development; no mycelia. Cultures from pus near testicle and cultures from the abdominal cavity gave the same mould that had been recovered from pleura and spleen of Case I. A culture from the blood remained sterile.

Result of *microscopic* examination:

Lungs.—Large areas in which the septa are moderately thickened by an infiltration with lymphocytes (mostly plasma cells). The air-spaces are partially collapsed. They contain enlarged and desquamated epithelial cells, some giant cells, in spots a few polymorphonuclear leucocytes and a little fibrin. *Parasites* were present in moderately large number, mostly mature forms. A few sporulating forms and some burst spore shells.

Inguinal Gland.—No changes.

Kidney.—No changes.

Spleen.—In the capsule small nodules consisting of cicatricial tissue with a few plasma cells in the periphery. *Many adult forms* in this tissue.

Heart.—A small thrombus between trabeculae carneae at the bottom of the left ventricle. In spots the thrombus shows irregular groups of polymorphonuclear leucocytes.

Cerebrum and *cerebellum* normal.

Testicle.—On the surface of the testicle has been formed an irregular thick layer of a rather cellular cicatricial tissue, in which there are few capillaries. In it there are small irregular patches of more cellular tissue. In spots these patches consist of epithelioid cells and contain giant cells of the Langhans type; in short, they resemble very much tubercles (Fig. 11). Sometimes giant cells containing parasites are directly imbedded in the cicatricial tissue. In most places, however, there are smaller and larger irregular abscesses which are in great part filled with polymorphonuclear leucocytes, and surrounded by a thin layer of epithelioid cells. Parasites are present in large number. The young and adult forms are mostly enclosed in the giant cells in tubercles and cicatricial tissue. Sporulating forms are found in the abscesses. The old sporulating forms have a rough outer surface. The minute irregular projections which one finds on their outside stain with Gram's method.

Diaphragm.—Large nodules on lower surface. Nodules consist largely of fibroblasts with small strands of connective-tissue fibrils between them. In spots there are groups of plasma cells. In one place there is a small nodule that consists almost entirely of fusiform cells with radial arrangement. The center of the nodule is formed by a *sporulating form* in which the spores are almost ready formed. In the fibrous tissue there are many adult *parasites*, mostly enveloped by a thin protoplasmic layer of large connective-tissue cells which frequently have several nuclei. Large typical giant cells are scarce.

Guinea-pig No. 3, male. March 2, 1900.

Inoculated with the pus from one of the testicles of Guinea-pig 2, intraperitoneally. In ten days there developed swelling of the testicles, later the symptoms subsided somewhat. The animal was killed April 7, with chloroform, and presented similar but more extensive lesions than the second guinea-pig. In addition to the nodules in spleen, diaphragm and lungs there were others in the peritoneum near the point of injection. The omentum was rolled up and transformed into a mass of hard white tissue with just visible yellow dots in it. Again the microscopic examination of fresh specimens showed the protozoon-like bodies in pure culture, but no mycelia, and tubes inoculated from testicle and omentum developed pure cultures of the mould.

Microscopic Examination.—*Omentum* is attached to pancreas by thick layer of dense cicatricial tissue. The omentum itself is transformed into an irregular mass of cicatricial tissue, which contains numerous abscesses. The cicatricial tissue is rich in cells; the majority of them are fusiform or more or less star-shaped connective-tissue cells. Between the cells there are bunches of connective-tissue fibrils. There are also many large polynuclear giant cells present, most of them of the Langhans type. In addition one finds a considerable number of lymphocytes. Apparently no capillaries.

The abscesses are filled almost entirely with polymorphonuclear leucocytes, but there are a few lymphocytes and a few irregular epithelioid cells in them. Of the latter there is a thin layer on the wall of the abscesses. In the centers of some of the abscesses one observes beginning disintegration of the polymorphonuclears, and beginning formation of fibrin.

Parasites.—Many young and adult forms are scattered through the cicatricial tissue, all enclosed in giant cells. Several show evidence of degeneration—a small amount of protoplasm, partial collapse of membrane (Fig. 17) and vacuolization. In the abscesses are only a few young and adult forms, but many burst spore shells. Of five sporulating forms, four were situated in abscesses. In those in which the spore membrane had burst, polymorphonuclear leucocytes have penetrated into the interior. In the sporulating form, which was not in an abscess, the protoplasm had divided, but the spores were not ready formed and the membrane was unopened. The small portions of protoplasm out of which the spores later form, stain very well, the spores themselves very badly, or not at all. No other bacteria, no mycelium.

The lesions in and around the *testicle* (Figs. 12, 13, 14, 16, 17) have very much the same microscopic appearance as those found in the omentum. The amount of suppuration is a little larger, and there is more evidence of destruction of polymorphonuclear leucocytes in the middle of the abscesses.

There is also a considerable amount of fibrin in these necrotic areas. Several *sporulating forms* and many burst shells are found in the abscesses. In the granulation and cicatricial tissue around the abscesses are several young and several adult forms (some of them in a degenerated condition) in giant cells.

Adrenal.—Normal.

Liver.—In one spot is a very small nodule in the periportal connective tissue. The nodule consists of fibroblasts and lymphocytes. There is one giant cell in it. *No parasites.* A complete series of sections from the nodule was, however, not obtained.

Lung.—A few small patches in which the septa are thickened by an infiltration with lymphocytes (mostly plasma cells) and fibroblasts. The air-spaces are collapsed. The epithelium is swollen and partly desquamated. In one of the air-cells several young *parasites* in a giant cell.

Diaphragm.—On lower surface a small nodule consisting of fibroblasts and mostly plasma cells. No *parasites* found (not a complete series).

Kidneys and *pancreas* normal.

Spleen.—One very small nodule consisting largely of epithelioid cells. It contains a few polymorphonuclear leucocytes and giant cells. No *parasite* found (not a complete series). In fresh specimens, however, a few adult forms were demonstrated.

Guinea-pig No. 4.

About 1 c.cm. of a suspension of a third generation from a pure culture of the mould on agar-agar was injected into the peritoneal cavity. The culture had been growing a comparatively short time only. The guinea-pig was killed about four weeks later. No lesions were found at the autopsy.

Guinea-pig No. 5. October 23, 1900.

An emulsion from a pure culture of the mould, which contained many spores, was injected subcutaneously. On November 2, marked swelling at the point of injection with central ulceration was noted. The ulcer was covered with a thick brown scab, underneath which there was a considerable accumulation of thick creamy pus. Killed December 7, 1900. The condition had not changed materially. There was little loss of weight.

At the postmortem, which was performed immediately, a small ulcer was found at the point of inoculation, which was covered by a thick brown scab. Underneath the scab there was a small accumulation of thick creamy pus. The bottom of the ulcer was formed by a thin layer of red granulation tissue. Underneath the granulation tissue there was some white cicatricial tissue. The muscles of the abdominal wall were not involved in the process. The inguinal glands were enlarged on the left side (side of inoculation). The largest one had the size of a small split-pea. In the center of this lymph-gland there was a comparatively large abscess-cavity which was filled with thick, light-yellow purulent material. In one place the abscess had perforated through the skin.

Spleen.—Slightly enlarged.

A few just visible nodules in the *lungs.*

The other organs did not show any microscopic changes.

In the pus from the ulceration a large number of protozoon-like bodies in different stages of development were found during the lifetime of the animal and also at the postmortem. Mycelia were not present.

Guinea-pig No. 6. October 23, 1900.

Inoculated with the same emulsion intraperitoneally.

November 2. Marked swelling of both testicles with reddening of the skin over them. The animal was killed on December 2. At the point of injection a small ulcer had formed, from which there was a scanty purulent discharge.

The tissues around the ulcer were transformed into a hard, partly greyish-white, partly yellowish mass. The change extends down to the peritoneum, which over the diseased area in the abdominal wall is considerably thickened, white in color and retracted in the form of a star-shaped scar. In the *parietal peritoneum* there are several projecting yellowish-white nodules. The largest one has the size of a millet-seed. The peritoneum near these nodules shows a white discoloration. Numerous nodules and extensive diffuse infiltrations are found in the diaphragm. One nodule with a small central defect is situated at the apex of the bladder.

The *omentum* is retracted and forms a thick, irregular, hard, nodular mass along the larger curvature of the stomach. On the left side it adheres to the abdominal wall. On cross section one finds in the partly white, partly greyish tissue many submiliary abscesses.

The *spleen* is adherent to posterior abdominal wall and laterally to the omentum. It is slightly enlarged and contains a few submiliary nodules.

In both *kidneys* there are a few greyish-white nodules.

Both *testicles* are about three times the normal size, and adherent to the skin. In front between testes and skin there are large, irregular abscess-cavities which are filled with thick creamy pus. On the left side the abscess has perforated. The testicular tissue is almost completely destroyed. In its place one finds greyish-white granulation and white cicatricial tissue.

In the *liver* there are a few nodules. The largest ones have the size of a millet-seed and show a central yellow discoloration. Many submiliary grey nodules in the *lungs*.

Heart normal.

A few small nodules in the *pancreas*.

On both sides of the spinal column there is a thick layer of granulation and cicatricial tissue in and underneath the peritoneum.

One *inguinal gland* on the opposite side from the point of injection is slightly enlarged.

Protozoon-like bodies were found in fresh specimens from the nodules and the abscesses. No mycelia.

Rabbit No. 1, large male. March 21, 1900.

Into vein of ear was injected .75 c.cm. of a milky suspension of a pure culture grown on sugar-agar-agar (2% glucose) with contained very many spores.

May 5. Rabbit much emaciated, very weak. Killed.

Very many submiliary white nodules in lungs, a few just visible nodules in spleen and kidneys.

Microscopic Examination.—Liver.—Very few, very small nodules consisting of epithelioid cells and lymphocytes with central giant cells. The nodules are situated in the periportal connective tissue.

Kidney (Fig. 22).—Submiliary nodules in which there is a gathering of epithelioid cells, some polymorphonuclear leucocytes and plasma cells in the spaces between the uriniferous tubules. Some such cells are also found inside of some of the tubules. In the center of the nodules the epithelial cells fill the lumen of the tubules completely and fuse together into large multinucleated protoplasmic masses. In some of these latter some of the nuclei have undergone degenerative changes; they shrink, become irregular in outline and stain very intensely throughout (pyknosis). Nevertheless they show a well-marked, very deeply staining nucleolus. The diseased areas are always situated near a small artery. There is a slight production of connective-tissue fibrils in them; no sign of caseation or suppuration. In some of the tubules one finds casts containing remnants of polymorphonuclear leucocytes and epithelioid cells. The nodules contain many young and adult *parasites*. Sporulating forms were not found in spite of careful examination of many sections, and fresh teased specimens. The protoplasm of most of the parasites is finely vacuolated. Their membrane is exceptionally thin. There are few degenerating forms and a few empty shells. In some places the parasites are situated in enlarged epithelial cells.

Spleen.—One small area consisting of fibroblasts with some connective-tissue fibers between them. There are also some lymphocytes (mostly plasma cells) and many large giant cells. Several young and adult *parasites* are found enclosed in the giant cells.

Lungs (Fig. 20).—Large nodular areas in which the septa are thickened on account of marked infiltration with lymphocytes (mostly plasma cells). The air-spaces are partly collapsed. The epithelium is swollen, and there are signs of proliferation. On account of the large size of the epithelial cells some of the air-spaces have a gland-like appearance. In other air-spaces the desquamated epithelial cells have become fused together into large giant cells. It is possible to recognize by the different appearance of their nuclei, that sometimes epithelioid cells also participate in the formation of such giant cells. In some of the giant cells some of the nuclei are pyknotic. In one especially large nodule the center is formed by detritus which is full of nuclear debris and remnants of polymorphonuclear leucocytes.

Parasites are present in moderate numbers; young and adult forms; no sporulating forms. Mycelia were not found in sections or in fresh teased specimens. No tubercle bacilli or other bacteria.

Rabbit No. 2. March 21, 1900.

Received .5 c.cm. of a suspension of a culture grown on beef-tea injected into large vein of ear. The culture apparently did not contain any spores. The animal died very much emaciated, May 17, 1900.

Many submiliary white nodules in lungs, kidneys, spleen. Near the free edge of the liver there are bright yellow, irregular, badly outlined spots with hyperæmic margins. Fresh teased specimens show protozoon-like bodies in all nodules; none were found in the discolored areas in the liver.

In some of the nodules two small forms are closely approximated and form figure-8 forms. In none of the specimens were any mycelia seen.

Microscopical Examination.—Heart.—A few subendocardial areas in which

there is a slight thickening of the connective tissue with atrophy of some of the muscle fibers. No parasites.

Intestine normal.

Spleen (Fig. 18).—Conglomerate submiliary nodules, consisting largely of epithelioid cells with many giant cells. In the centers of some of them there are small collections of polymorphonuclear leucocytes, some of which show karyorrhexis and beginning disintegration of the protoplasm. They are imbedded in a stringy material which, by van Gieson's method, stains dark brown, with eosin bright red (fibrin). In the peripheral parts of the nodules are many lymphocytes (mostly plasma cells). In the periphery of nodules connective-tissue fibers are found between epithelioid cells.

Parasites.—Moderate number. Mostly in giant cells, adult forms; many collapsed, degenerating forms with little protoplasm or none at all. No bacteria.

Adrenal.—Normal.

Kidney.—Nodules very much like nodules in spleen; perhaps more lymphocytes throughout nodules; marked evidence of formation of fibrous tissue in periphery.

Parasites.—None found in sections, but a few in each nodule in fresh teased specimens. No bacteria.

Liver (Fig. 15).—Extensive necroses of spherical or irregular form (yellow spots). The spaces between the necrotic liver cells are filled with very irregular epithelioid cells. In the central portions there are many polymorphonuclear leucocytes in varying stages of disintegration and much nuclear debris. The necrotic liver cells stain intensely red with eosin. With van Gieson's method the peripheral ones stain light brown very much more like normal liver cells; the central more degenerated ones more darkly. In the periphery of the nodules a few lymphocytes (mostly plasma cells).

Parasites found neither in sections, nor in fresh teased specimens; no bacteria.

In other parts of the liver there are submiliary nodules consisting of epithelioid cells (Fig. 19).

Lung.—Single or conglomerate nodules where the septa are thickened and air-spaces are filled with cells mostly of the epithelioid type. In peripheral parts of nodules one finds connective-tissue fibers between the epithelioid cells. With the epithelioid cells there are found lymphocytes (mostly plasma cells) especially in the periphery of the nodules. In the central portions of some of the larger nodules there are accumulations of polymorphonuclear leucocytes surrounded by necrotic material which stains very intensely with eosin and dark brown with van Gieson's method. In one place a microscopic cavity has been formed by the destructive process. The septa of the adjoining air-spaces are slightly thickened, the air-spaces themselves are collapsed and either partly or entirely filled with large desquamated epithelial cells.

Parasites are present in considerable numbers, mostly young or adult forms. Many exhibit signs of degeneration. They are folded, contain little or no protoplasm, or show vacuolization. In one place are a few sporulating forms surrounded by polymorphonuclear leucocytes. All other forms are enclosed in giant cells.

Uterus and *cerebrum* normal.

Rabbit No. 3, large male. March 21, 1900.

Received .2 c.cm. of a suspension of a third generation of the mould (mycelium with few spores) injected into large vein of ear.

August 2. The animal shows complete paralysis of posterior part of body. Killed.

Post-mortem Findings.—Peritoneal cavity.—No abnormal contents. *Bladder* very much distended, reaches almost to lower surface of liver. Numerous white slightly retracted scars of the size of a pin's head in the *spleen* and kidneys. A few white nodules of the same size in posterior part of *liver.* Larger white bard nodule of the size of a millet-seed on the lower surface of the right half of the *diaphragm.* Just visible greyish-white nodules in both *lungs. Heart* normal. In the serosa over the large intestine is a miliary white, hard, projecting nodule. *Brain.*—After removal of skin covering the head, the bone is found to project about 2 mm. above the normal level on both sides over the frontal lobes. The projection is more marked on the left side. The projecting area measures about 3 mm. in diameter and is of a yellowish color with red margin. Underneath it there is an abscess filled with thick semi-fluid pus. On the lower surface the abscess projects into the skull and has caused considerable compression of the left frontal lobe. Brain and meninges otherwise normal. The lower part of the lumbar cord is compressed by a tumefaction and purulent infiltration of the posterior parts of three of the upper lumbar vertebrae and the adjoining tissue.

Examination of fresh specimens.—In the abscess over the left frontal lobe there are a moderate number of parasites; adult forms, some empty shells, three sporulating forms. Otherwise the abscess contains mostly fatty detritus. Similar findings are noted in specimens from the diseased area in the lumbar region, but the specimens contain less fat.

In the nodule in the *lung,* a few adult forms are seen; there is much fat in central part of nodule.

The nodule in the liver is calcified; there are no parasites.

The nodule in the kidney shows one empty shell.

The nodule in the spleen shows several adult and young forms. One parasite shows a figure-8 form. In one spot about eight young forms are enclosed in a common capsule.

The diaphragmatic nodule is filled with pus, which contains many adult and young forms, two sporulating forms, one group of young forms in a common capsule, and several parasites with figure-8 form.

Microscopic Examination.—Spleen.—The capsule shows a slight wrinkling. At the bottom of the depressions there are small masses of fibrous tissue, which differ from the connective tissue of the capsule by an absence of involuntary muscle-fibers, and also by an irregular infiltration with lymphocytes. No *parasites* found in sections.

Liver.—In most places there is a slight (in some a marked) proliferation and moderate lymphocytic infiltration of the periportal connective tissue. Some of the fibroblasts have entered the spaces between the rows of liver cells and are beginning to encroach upon the latter. No nodules; no parasites.

Kidney (Fig. 21).—In the cortex and medulla are seen irregular small areas in which the uriniferous tubules are small. The epithelium in them is

small, not differentiated. Between the collapsed tubules there are strands of fibrous tissue. The glomeruli in these areas show a fibrous thickening of the capsule; in others is seen typical hyalin degeneration. Quite a number of the uriniferous tubules inside and near such areas contain large hyalin casts. The lymphatics in the medullary part of the kidney are very much distended and filled with hyalin material (coagulated lymph?). No *parasites* in sections.

Lung.—The nodules of the lung appear in two different forms. In one the center of the nodule consists of irregularly arranged large cells with a large spongy protoplasmic body. With such cells one finds irregular groups of lymphocytes. The periphery is made up of dence fibrous tissue. These nodules contain a few adult intact or degenerated *parasites*. The other nodules consist of fibrous tissue in which the remnants of the air-spaces appear as gland-like formations with cuboid epithelial cells on their wall. No *parasites* found in such nodules. One of the larger branches of the pulmonary artery is completely obstructed by a thrombus. The thrombus consists of conglutinated blood platelets, red blood corpuscles, some fibrin and in the middle of it there is considerable nuclear debris. There is no evidence of organization. No *parasites*.

Intestine.—The nodule situated in the subserosa has destroyed the muscularis but does not reach into the mucous membrane. The periphery of the nodule is formed by dense fibrous tissue with slight lymphocytic infiltration. In the middle of the nodule there is an irregular necrotic area which is composed of granular albuminous detritus, and nuclear debris in large quantity. There are also a few polymorphonuclear leucocytes and a considerable number of very large irregular cells with large clear nuclei, the protoplasm of which contains remnants of cells and nuclear debris. Interposed between the peripheral and central parts of the nodule one finds a thin layer of granulation tissue composed of large epithelioid cells, lymphocytes, a few polymorphonuclear leucocytes, and some large giant cells. *Parasites.*—Many adult forms are seen in the granulation tissue, mostly in giant cells. In the broken-down centers of the nodules are some adult forms, many empty shells, several sporulating forms. In one spot is seen a shell with about six young forms inside it.

Spinal column.—In the bony parts laterally and posteriorally the medullary tissue is replaced by granulation tissue; many of the remaining bony spicula show evidence of beginning absorption (Howship's lacunae with osteoclasts). In the granulation tissue there are numerous submiliary abscesses, that are either filled almost entirely with polymorphonuclear leucocytes or show polymorphonuclear leucocytes in the periphery and in the center a necrotic mass that consists of albuminous detritus, remnants of cells and nuclear debris. In spots the granulation tissue around these abscesses shows extensive necroses. These necrotic areas contain less nuclear debris. The granulation tissue is in most places surrounded by a layer of cicatricial tissue. There is very little, if any, new formation of blood-vessels. There are comparatively few giant cells in the granulation tissue.

Parasites.—In the granulation tissue are many adult forms, some in giant cells. In many of the abscesses sporulating forms are seen. The spores are

somewhat different in form. In the middle of the mass of spores one usually finds S-shaped forms. Laterally they are mostly biconcave disks or biconcave plates, some three-cornered, other hexagonal. There are also pyramidal forms with concavities on all their surfaces. Of all forms only the peripheral ones stain at all; quite well with hematoxylon or other nuclear dyes, whereas eosin and other protoplasmic dyes stain them less distinctly.

Skull (Fig. 24).—Lesions of the same character, by which in one place the bone is destroyed in its entirety from dura to outer periosteum.

Rabbit No. 4. September 10, 1900.

Small white rabbit inoculated with 1 c.cm. of a milky suspension of culture, containing many spores, into ear-vein.

September 12. A small abscess has formed at the point of injection.

Microscopic examination of the pus shows many polymorphonuclear leucocytes, many spores. A few of the spores have germinated and developed into short hyphae (Fig. 28).

September 13. Hyphae disappearing. Many of the spores are developing into protozoon-like bodies (Fig. 29). The development takes place by simple enlargement. Either the spore grows as a whole or there is first a bulging out on one side with thinning of the spore-membrane at that point. The spore membrane, however, develops directly into the membrane of the protozoon-like bodies.

September 14. Further development of protozoon-like bodies. One finds already several sporulating forms. In one place there is an empty shell. This shell and several more of the later stages are distinctly prickly on the surface (fresh specimen without addition of any chemical). One form has a figure-8 shape. One body is not spherical, but has a decided pear-shape.

September 15. Very many sporulating forms; very many forms with prickly surface.

September 17. Hardly any mould-spores left. Many empty shells. Many of the adult forms show a large central or slighly eccentric vacuole (Fig. 30); one of them has a distinct pedicle (Fig. 30).

September 20. Some pus, which at this time does not contain any of the original spores from the mould, is suspended in a drop of beef-tea, and incubated for 24 hours. After that time numerous hyphae have developed from adult forms (Figs. 32 and 33) and also from spores of protozoon-like bodies (Fig. 34). The hyphae are especially numerous around sporulating forms.

The animal died in the night from October 15 to 16, 1900.

Post-mortem examination, October 16, 9 A.M.

The animal is very much emaciated. At the *site of inoculation* there is a swelling, which extends about 2 cm. from the basis of the ear towards the distal end following the marginal vein. The swollen parts are covered with thick scabs, underneath which there are irregular ulcerations in the skin. The swollen tissues are irregularly infiltrated with pus.

Lungs.—Bright red. Very many, just visible, grey nodules are seen.

Spleen.—Slightly enlarged. A few just visible nodules are recognized.

Kidney.—A few just visible subcapsular nodules. Many larger ones (about the size of millet-seeds) with grey periphery and yellow center projecting over cut surface at the border line between cortex and medulla.

Liver.—No macroscopic lesions.

Bladder.—Much distended.

A few drops of slightly bloody fluid in the peritoneal cavity.

No lesions in the central nervous system.

Experiments on *cold-blooded* animals have been confined to two *frogs*. One was inoculated subcutaneously, the other into the peritoneal cavity. In both experiments the result was absolutely negative.

Collodion sac experiment.—August 25, 1900, a collodion sac containing a little beef-tea and some material from a pure culture with many spores was introduced into the abdominal cavity of a rabbit. August 30. Rabbit killed; some suppuration around sac. Contents consist entirely of spores; no protozoon-like bodies.

EXPLANATION OF PLATES.

(*Figs.* 1–24 *inclusive are microphotographs.*)

PLATE XXXIV.

Fig. 1. × 90. Tubercle-like nodule in liver of first human case.

Fig. 2. × 90. Necroses and abscess-formation in lungs of first human case. In center one sporulating form.

Fig. 3. × 50. Showing general distribution of diseased process in lungs of first human case.

Fig. 4. × 90. Lungs of first human case. Air-spaces filled with granulation tissue consisting of epithelioid cells and lymphocytes. In the center are adult parasites enclosed in a giant cell.

Fig. 5. × 90. Two abscesses containing sporulating forms in the kidney of first human case.

Fig. 6. × 90. Tubercle-like nodule around a glomerulus in the same kidney.

PLATE XXXV.

Fig. 7. × 90. Tubercle-like nodule in pericardium of second human case. In center large giant cell containing adult forms of parasite.

Fig. 8. × 90. Small abscess containing sporulating forms in spleen of first human case.

Fig. 9. × 40. General view of lesions in meninges of second human case.

Fig. 10. × 90. Lesions in meninges of second human case. Higher power. In center small abscess with empty spore shell.

Fig. 11. × 90. Tubercle-like nodule with giant cell and central caseation. Many young and adult parasites. Testis of Guinea-pig No. 2.

Fig. 12. × 17. General view of lesions of testis of Guinea-pig No. 3. Tubercle-like nodules and irregular abcesses, both imbedded in cicatricial tissue.

PLATE XXXVI.

Fig. 13. × 340. Testis of Guinea-pig No. 3, showing parasite in beginning of sporulation and empty spore-shell full of polymorphonuclear leucocytes.

Fig. 14. × 90. Testis of Guinea-pig No. 3. Small abscess containing sporulating forms.

Fig. 15. × 90. Liver of Rabbit No. 2. Extensive necrosis with much nuclear debris and some polymorphonuclear leucocytes in periphery.

Fig. 16. × 340. Testis of Guinea-pig No. 3, showing sporulating form that has recently burst. A polymorphonuclear leucocyte has just entered through rent in capsule.

Fig. 17. × 340. The same. Showing granulation tissue with giant cells and adult forms of parasite. One parasite shows folding of capsule.

Fig. 18. × 90. Spleen of Rabbit No. 2.

PLATE XXXVII.

Fig. 19. × 90. Another part of liver of Rabbit No. 2. Tubercle-like nodule in periportal connective tissue. Very early stage.

Fig. 20. × 90. Lung of Rabbit No. 2. Formation of large giant cells by fusion of desquamated epithelial cells. In one of them an adult parasite.

Fig. 21. × 90. Healed lesion in kidney of Rabbit No. 3.

Fig. 22. × 90. Kidney of Rabbit No. 2.

Fig. 23. × 90. Diaphragm of Guinea-pig No. 2, showing plasma cells on left side; many adult forms; encapsulated sporulating forms in tubercle-like nodule (has been slightly retouched in order to show the sporulating parasite better).

Fig. 24. × 10. General view of lesion of skull in Rabbit No. 3.

PLATE XXXVIII.

Fig. 25. Sporulation of pathogenic mould in potato culture, kept at room temperature twenty days. Spores about 8 μ in largest diameter.

Fig. 26. Gilchrist's mould. Sporulating (?) forms. Potato culture kept at room temperature for twenty days. Bodies vary from 5–9 μ in diameter.

Fig. 27. The same.

Fig. 28. Germination of spores of mould in abscess of ear of Rabbit No. 4, 2nd day.

Fig. 29. Development of protozoon-like bodies from spores of mould in abscess of ear of Rabbit No. 4, 3rd day.

Fig. 30. Unusual form of parasite found in abscess of ear of Rabbit No. 4, 5th day.

Fig. 31. Germination of spores of mould when transferred to new medium (gelatine drop, kept at room temperature for 48 hours). The spore membrane always bursts at the side.

Figs. 32 and 33. Development of mycelium from protozoon-like bodies when kept in artificial media (hanging drop of beef-tea kept for 24 hours in incubator).

Fig. 34. Development of mycelium from spores of protozoon-like bodies.



FIG. 1.

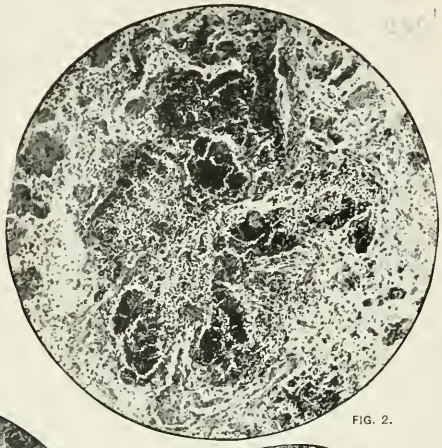


FIG. 2.



FIG. 3.

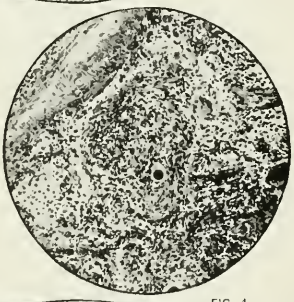


FIG. 4.



FIG. 5.

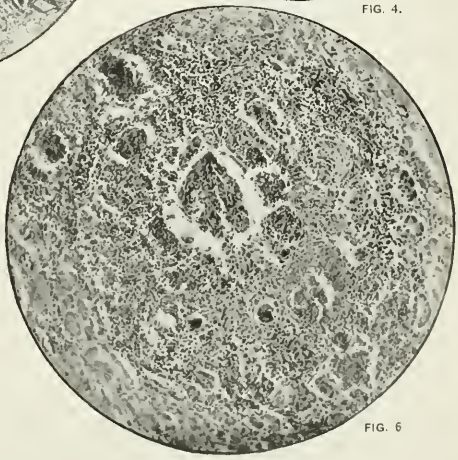


FIG. 6

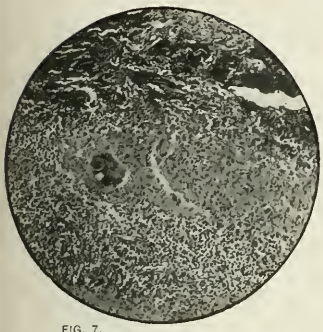


FIG. 7.

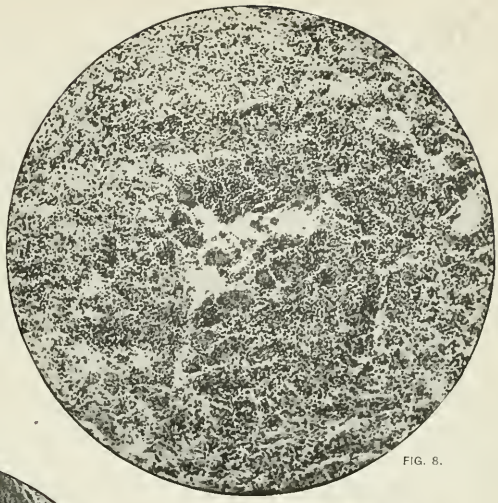


FIG. 8.



FIG. 9.



FIG. 10.

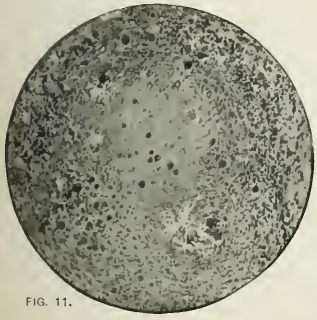


FIG. 11.



FIG. 12



FIG. 13.

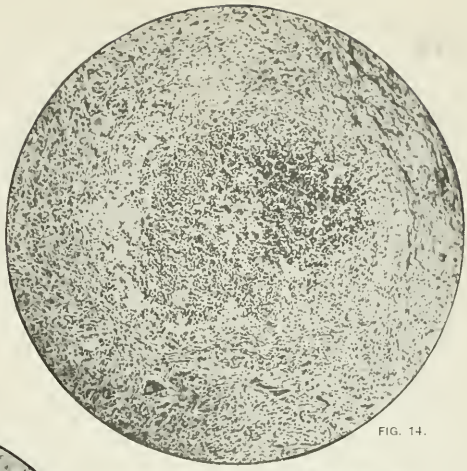


FIG. 14.

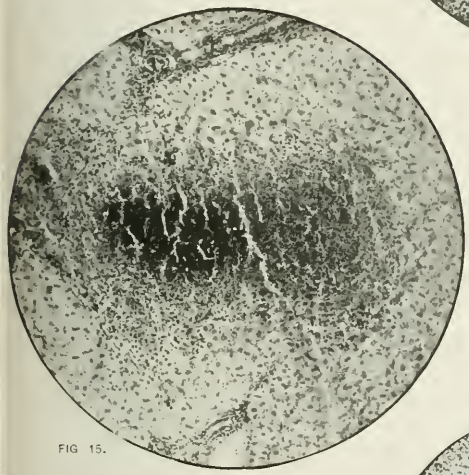


FIG. 15.



FIG. 16.

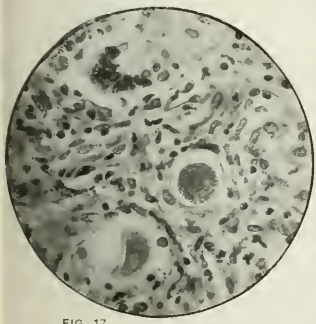


FIG. 17.

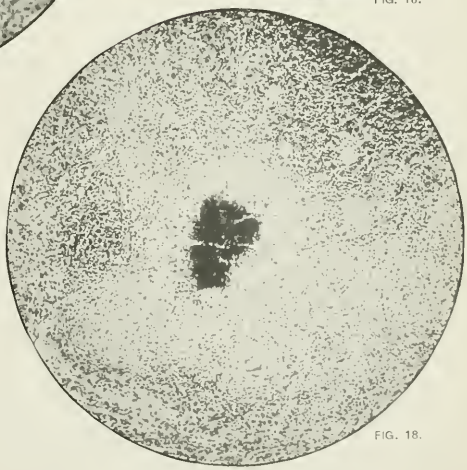


FIG. 18.

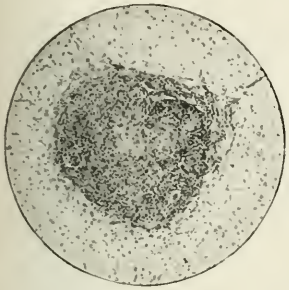


FIG. 19.



FIG. 20.

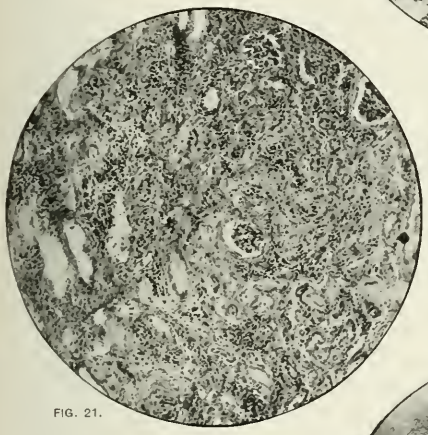


FIG. 21.



FIG. 22.

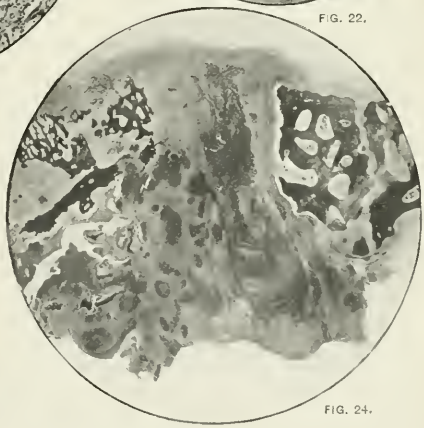


FIG. 24.

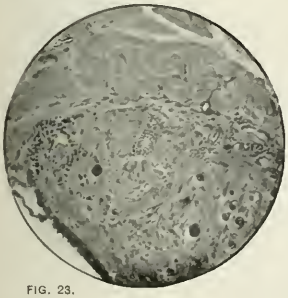


FIG. 23.



FIG. 25.

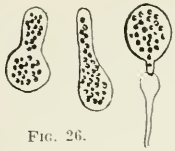


FIG. 26.



FIG. 27



FIG. 28.



FIG. 31.



FIG. 29.



FIG. 30.



FIG. 34.

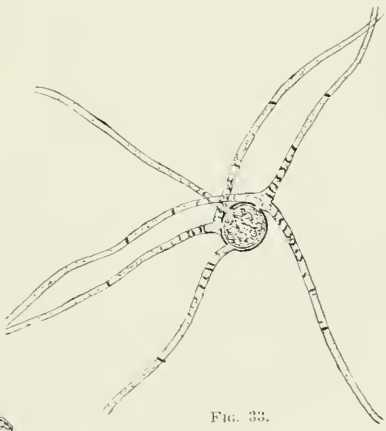


FIG. 33.



FIG. 32.

THE TOXIC EFFECTS OF FORMALDEHYDE AND FORMALIN.

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Although the germicidal effects of formaldehyde and formalin have been in the last few years the subject of much discussion, yet the study of the action of these substances upon the animal economy has been practically neglected. Much of the literature on this subject is of little value because supported by insufficient experimental evidence, and because overhasty conclusions have been drawn. In the following pages I have collected the literature which bears most directly upon the subject of my paper. Wherever the term *formaldehyde* is used in this article, the gas formic aldehyde, CH_2O, is referred to. By the term *formalin* is meant a 40% solution of formaldehyde in water. The percentage of solutions relates to the amount of *formalin* in the solution. Thus a 10% formalin solution contains 4% of formaldehyde.

In the experiments, immediately after the death of an animal, brought about naturally, or induced by chloroform, ether or a blow on the head, the tissues were fixed in Zenker's fluid; in a few instances alcohol was used. In staining, eosin and haematoxylin were employed, supplemented when necessary by staining for fat with Sudan III, by Weigert's fibrin stain, and by polychrome methylene blue. When no special stain is designated, the description refers to the picture shown by eosin and haematoxylin.

The fixing action of formalin itself is to be remembered; and also that in consequence, a cell killed by contact with the chemical may histologically show no evidences of death.

I. CHANGES IN THE LUNGS AFTER INHALATION OF FORMALDEHYDE.

The following experiments were performed in a room of 5.5 cubic metres capacity. A large door opening into the room was closed but

no attempt was made to prevent the egress of the formaldehyde or the ingress of air through the keyhole, cracks in the door, etc. Formaldehyde gas was generated by slowly volatilizing paraformaldehyde pastils in a Schering disinfecting lamp. The lamp was set in the middle of the room, the animals being in a cage near the door. In all, nine animals were used.

Exp. 1.—A guinea pig and a rat were exposed for 1½ hours; 3 grm. paraformaldehyde volatilized.

Exp. 2.—A guinea pig and a rat were exposed for 3½ hours to an atmosphere in which 5 grm. of paraformaldehyde had been volatilized.

Exp. 3.—A guinea pig and two rats were exposed for 6 hours to an atmosphere in which 11 grm. of paraformaldehyde were slowly volatilized.

When removed, the animals were slightly dazed but otherwise apparently well. At post-mortem, forty-two hours later, the lungs of the animals were somewhat reddened. In the third experiment crepitation was slightly diminished in the lungs of all three animals. The bronchi contained a small amount of mucus.

Cultures were made from the nose, mouth, throat and lungs of each of the animals. In three of the animals micrococcus tetragenus was isolated from the mouth. A single colony of the same bacterium was obtained from the lungs of one of the guinea pigs. All the other cultures were sterile.

Histological examination reveals distinct inflammatory changes in the lungs of the animals. The lungs of the rat and guinea pig exposed for one and a half hours show marked congestion. Many of the alveoli are filled with a homogeneous red-staining exudate. Slight desquamation of the alveolar lining cells has occurred. Many polymorphonuclear leucocytes are found in the capillaries, in the connective tissue of the lung and free in the alveoli. The eosinophiles are numerous (Fig. 1). Mononuclear leucocytes are present but are few in number. The bronchi are filled with serum containing a few leucocytes and desquamated and degenerated bronchial epithelium. The deeper layer of the cells of the bronchi are still

attached to the basement membrane. In the peribronchial connective tissue many leucocytes are found. Numerous red blood corpuscles are mixed with the leucocytes in the alveoli and bronchi. The trachea shows no change.

In the two animals exposed for three and a half hours, the changes are more severe. The most striking difference is noted in the character of the leucocytic infiltration. Localized areas of pneumonia are found involving either a single alveolus or several alveoli; often these are peribronchial or perivascular (Fig. 2). The major part of the leucocytes which have accumulated are of the mononuclear variety. The polymorphous variety has also increased but not in proportion to the mononuclear variety. The number of leucocytes and alveolar epithelial cells in the bronchi is larger than in the foregoing experiment.

In the three animals exposed for six hours, the pneumonia is similar to that just described, but the process is more diffuse and involves large areas of the lung substance. A beginning tracheitis is found in the animals exposed for three and a half hours (Fig. 3), but in the last series of experiments this is more marked. The blood-vessels of the submucosa are congested and leucocytic infiltration has occurred. The latter is mainly eosinophilic. Many leucocytes are found between the epithelial cells lining the trachea. The epithelial cells themselves show slight degenerative changes, and are often desquamated.

Exp. 4.—A spaniel, wt. 25 kilo., was left for 5 hours in the before described room. The formaldehyde lamp was set on a high shelf and 5 grm. of paraformaldehyde volatilized in the course of the first half hour. The door of the room was opened several times. The slight dry cough present when the dog was removed from the room became moist in character several hours later. On the following day the animal coughed incessantly; and this coughing continued until the third day when the dog was bled to death.

Exp. 5.—A large cat was allowed to run about in the room while 250 cc. of methyl alcohol were oxidized to formaldehyde in a Moffat lamp. Time, 2¾ hours.

At autopsy the lungs are less crepitant than normally, œdematous, and float low in water. Small areas of consolidation are scattered through the lung substance. The bronchi are filled with a greyish, creamy mucus. The trachea is covered with mucus.

By histological examination a diffuse pneumonia, with here and there areas of more intense infiltration, is found to be present in the lungs of both animals. There is general vascular congestion. In the dog the blood-vessels contain an enormous number of eosinophiles and other polynuclear leucocytes. Only rarely can a mononuclear leucocyte be found. In the cat the polymorphonuclear leucocytes are much less in evidence, and the mononuclear forms are predominant. Many of the alveoli are filled with a homogeneous red-stained exudate. This is most marked in the cat in which over half of the alveoli are filled. The leucocytes are mainly of the polymorphous variety, and are most numerous in the walls of the alveoli. The alveoli themselves contain leucocytes and large epithelioid cells which probably represent desquamated alveolar epithelium. The bronchi are filled with large numbers of leucocytes, broken down bronchial epithelium, and granular detritus. The superficial lining cells of the bronchi are swollen and stain poorly; the cellular reticulum is loosened and the cells have lost their sharp contour. The nuclei are swollen and stain palely. Between the cells are found many leucocytes. Desquamation is common, and, when severe, the submucosa is entirely uncovered. In the submucosa and in the peribronchial connective tissue large numbers of leucocytes are found. A more severe bronchitis is found in the dog than in the cat.

It can at once be seen that my observations are directly opposed to those of Aronson, Pfuhl, Rosenberg, Fairbanks, Grawitz, Babes, de Schweinitz, Kobert and Moeller, all of whom conclude that formaldehyde produces no effects upon the animal economy.

Aronson (1) subjected several white mice and a rabbit to the vapors of formaldehyde and found, after twenty-four hours, that the animals were alive and well. A histological examination showed no tissue changes. Fairbanks (2) found the same true of rabbits and mice

which he had subjected to formaldehyde inhalation for twenty-five hours. De Schweinitz (3) kept a calf in a 2% atmosphere of formaldehyde for five hours, and beyond a slight watering of the eyes and an occasional cough, could find no deleterious effects. Kobert (4) found that guinea pigs permitted to run loose in a room during formaldehyde disinfection did not become sick. Moeller (5) allowed six guinea pigs to run about in rooms which were disinfected upon four consecutive days. After that time he found the animals lively and well and they remained perfectly well thereafter. Doty (6) could find no tissue changes in insects, fowls, guinea pigs, mice, etc., subjected to formaldehyde gas for periods of from three to fifteen hours; occasionally a guinea pig would show a slight inflammation of the respiratory tract.

Klipstein (7) allowed two rabbits to inhale formaldehyde gas from a heated formalin solution. The first of these inhaled the gas for one-half hour; the other for one-half hour on three consecutive days. The post-mortem examination showed rhinitis, laryngitis, tracheitis and catarrhal bronchitis. These changes were more severe in the second animal. A muco-purulent material was found in the bronchi. The lungs were normal. Except for the last statement these findings agree with those in my experiments.

Harrington (8) has found that cats and dogs accidently confined in rooms undergoing disinfection rarely survive the process. As proof he cites two experiments of his own, and refers to the experience of several persons daily engaged in house disinfection. Likewise, Francis, of the health department of St. Louis, Mo., several times put rats into rooms undergoing disinfection. Except in one instance they were all killed in one and a half hours. Two guinea pigs, likewise experimented upon, remained well. The death of the investigator Staub has been attributed to the inhalation of formaldehyde.

Schlossman has worked with a combination of glycerine and formalin (glyco-formol) and urges the necessity of wearing a gas mask when entering a room in which disinfection has been performed.

II. INJECTION OF FORMALIN INTO THE STOMACH.

Formalin was injected directly into the stomach by means of a soft rubber catheter introduced through the oesophagus. The symptoms following the injection of formalin differed in the different animals. In all, eleven animals were used.

Exp. 1.—Rabbit, wt. 500 grm. Injected 8 cc. of concentrated formalin. The animal falls upon its side and expires in five minutes.

Exp. 2.—Rabbit, wt. 500 grm. Injected 4 cc. of 10% formalin. The animal died immediately.

Exp. 3.—Rabbit, wt. 510 grm. 6 cc. of 10% formalin were injected. The animal died in one minute.

Exp. 4.—Rabbit, same wt., size and age as preceding. Injected 6 cc. of 10% formalin. The rabbit loses weight rapidly and dies in three and a half days.

Exp. 5.—Kitten, wt. 902 grm. Injected 5 cc. of 10% formalin. Catheter was bitten in half on withdrawal, and a few drops of the formalin got into the mouth. Death ensued in one hour.

Exp. 6.—Kitten, same wt., size and age as preceding. Injected 1 cc. of 10% formalin. On the following day the kitten has developed an intensely foul breath which continues to the day of death, three and a half days later.

Exp. 7.—Kitten, wt. 1021 grm. Injected 3.5 cc. of 10% formalin. Death occurs on the ninth day after the injection of the formalin.

Exp. 8.—Rabbit, wt. 1380 grm. Injected 8 cc. of 5% formalin. Death occurs in six days.

Exp. 9.—Terrier, wt. 53 kilo. Injected 17 cc. of 5% formalin. After a minute the dog vomited a frothy mucus which had a strong odor of formalin. After half an hour the animal becomes quiet. Death occurs in forty hours.

Exp. 10.—Kitten, wt. 980 grm. Injected 3 cc. of 10% formalin. The kitten is killed three weeks after the operation, in which time it has grown and gained in weight.

Exp. 11.—Rabbit, wt. 1401 grm. Injected 6 cc. of 5% formalin. The animal ate and increased in weight. After a month it was killed.

On post-mortem examination no macroscopic changes except a reddening of the mucous membrane of the stomach and duodenum are

found in the animals that died immediately after the injection of the formalin. Histologically, only an intense vascular congestion exists.

The stomach of the rabbit in the fourth experiment contains only a few drops of a turbid liquid. The mucous membrane is grayish, soft, and can be easily scraped off; the pyloric end of the stomach is ulcerated. The intestinal tract is empty, except the caecum, which is filled with a soft, brownish, gruel-like material.

Histologically, the serosa and the muscularis show no change. The submucosa has undergone loosening so that the connective tissue fibrillæ are widely separated from each other. The blood-vessels are markedly congested, but no haemorrhages have occurred. There is only a slight leucocytic infiltration. An increase in the staining reaction of the gland cells is the most striking change noted in the mucosa: all the cells are more deeply colored with haematoxylin than normally, and in the late stages of degeneration they stain a uniform, deep purple. The changes are more severe upon the rugæ than in the tissue between them. In the latter locality the cells are swollen and granular. The outlines of the cells are lost and the nuclei are irregular in shape. The peptic cells are swollen, granular, and stain red with eosin. Upon the rugæ the glandular cells are contracted. Many of the nuclei are lost, while those remaining are misshapen beyond recognition. Desquamation of the gland cells is everywhere present. At the top of the rugæ desquamation of all these cells has occurred, so that the gland cells are represented by a deep purplish-staining detritus within the connective tissue casing of the tubules. Many of the tubules are widely distended with necrotic matter. In some places only the intertubular connective tissue is found remaining. Areas are found in which a total loss of the mucosa has taken place, exposing the bare submucosa beneath. The large ulcer seen with the naked eye is found to be due to such a loss of the mucosa.

The stomach of the dog in Exp. 9 contained a few cubic centimeters of a turbid fluid similar to that found in the stomach of the rabbit just described. The mucous membrane was similarly gray and soft.

The histological findings are similar to those previously described, but the more deeply stained glandular cells extend only half way down the tubules. The cells are separated from each other, and a well-marked desquamation is present. A marked infiltration with polymorphonuclear and large and small mononuclear leucocytes has occurred. This is confined to the mucosa and submucosa. The changes, gradually diminishing in severity, extend through the duodenum into the small intestine for the distance of about a foot.

The kitten in Exp. 5, which died an hour after the injection of the formalin, showed the following changes on post-mortem examination. The mouth, pharynx and posterior nares are red and covered with mucus. The mucous membrane of the oesophagus is reddish in color and soft; and can be scraped off leaving a raw surface studded with red points. The stomach is filled with food from which emanates a strong odor of formalin. The mucous membrane is soft and reddish. All the vessels are markedly congested. Similar changes are found in the duodenum.

Histological examination shows a marked vascular congestion of the tissues of the mouth, pharynx and oesophagus. The connective tissue elements of the oesophagus are loosened so that the fibrillæ are widely separated from each other. No changes can be found in the epithelial cells. The congestion of the stomach and duodenum is very marked; in the latter location the separate capillaries can be traced to the tip of the villi. The glandular elements show no change. An acute oedema, manifesting itself by exudation into the submucosa, has occurred and in consequence the stomach wall is the seat of an immense bleb. The exudate makes up about half the thickness of the wall, and is stained homogeneously red. There is no leucocytosis.

In the stomach of the kitten in Exp. 6 are found 4 cc. of a dirty, turbid fluid. The mucous membrane is grayish, soft and can be scraped off easily. The stomach of the kitten in Exp. 7 is filled with a soft, pus-like material. The mucous membrane is grayish and soft; in some parts it has ulcerated. The mucous membrane of the

duodenum has a similar character. The gall-bladder is enormously distended with bile, and a bristle can with difficulty be pushed through the bile duct.

The microscopic findings in these two experiments are quite similar. In both cases we have to do with an intense gastritis. In the first of them the leucocytosis is most severe in the mucosa; the submucosa is also infiltrated with leucocytes, but in the muscularis only a few are seen. In Exp. 7 the inflammatory process involves the muscular tissue especially in those parts where the mucous membrane has ulcerated. The leucocytes present are mainly of the mononuclear variety; only a few polynuclear leucocytes are seen. The gland cells of the superficial parts of the mucosa are in various stages of disintegration. The infiltrating leucocytes show karyolysis and karyorrhexis. A granular, violet stained, necrotic material, mixed with mucus and broken down nuclei is found lying upon the mucosa. The mucosa and submucosa of the duodenum in Exp. 7 is the seat of marked inflammatory changes. This probably brought about an atresia of the gall duct, and a consequent distension of the gall-bladder with bile.

By far the most interesting series of pathological changes are found in the rabbit of Exp. 8. The stomach contains about 8 cc. of a foul-smelling, brownish, gruel-like liquid. The mucous membrane is deeply red, mottled with smaller areas of white, and traversed by thick, firm ridges. The whole is covered by mucus mixed with a whitish necrotic material; when the hand is brushed over the lining of the stomach the mucous membrane is scraped off, and a raw surface studded with red points is left.

On histological examination the changes in the stomach wall are found to be irregularly distributed and of varying intensity. Desquamation of the serosa has occurred in some places. The connective tissue of the subserosa retains a distinct bluish tinge after staining with haematoxylin. The muscularis shows changes ranging from a slight inflammatory condition to total necrosis. In those regions in which the muscular coat is least affected the muscle cells are slightly

swollen and stain palely. Many of the muscle cells are entirely separated from their bundles, and are in various stages of degeneration. With these changes is found an infiltration of polynuclear and mononuclear leucocytes. The muscular layer is affected first on the side nearest the mucous membrane, whence the changes progress downward until the serous side of the muscularis is reached.

The submucosa is the seat of an enormous fibrinous exudate which varies in thickness in different parts of the sections; at one time the layer of fibrin is scarcely recognizable, at another it is three times as thick as the normal wall. The ridges mentioned in the anatomical description are entirely due to submucous fibrinous exudation. The exudate, especially in its deeper portions, contains many polymorphonuclear leucocytes; some mononuclear leucocytes are also found. The muscularis mucosæ shows changes similar to those of the muscularis. The mucosa shows a series of changes; in the least affected parts only a few swollen cells are found, but in the more altered parts there is slight desquamation and necrosis accompanied by leucocytic infiltration. The entire thickness of the mucosa is at times wholly destroyed; it is then found lying upon its basement membrane as a necrotic mass, stained in various ways from a light red to a deep purple. Large areas of haemorrhage into the mucosa and underlying fibrinous exudate are found; the blood-vessels are enormously distended and in places the walls of ruptured vessels are seen.

The stomachs of the animals in Exp. 10 and Exp. 11 were filled with food; and no macroscopic or microscopic changes were demonstrable.

The wide range in the character of the pathological changes has forced me to describe these experiments in detail. Briefly summarized, the histological findings in the eleven stomachs examined are as follows:

Intense vascular congestion in Exp. 1, 2 and 3.

Infiltration of the mucosa with leucocytes together with necrosis of gland cells in Exp. 4 and 9. The necrotic material is hyaline and stains deep purple with haematoxylin.

Intense vascular congestion with acute oedema of the submucosa in Exp. 5.

Gastritis involving the entire wall of the stomach, and characterized by marked leucocytosis, karyorhexis, and necrosis in Exp. 6 and 7. The necrotic material is granular and stained violet with haematoxylin.

Marked leucocytosis and necrosis in all the tunics of the stomach, together with an intense fibrinous and haemorrhagic exudation into the submucosa in Exp. 8.

No recognizable changes in Exp. 10 and 11.

It is evident that I failed in my attempt to get a series of experiments which would show a progressive increase in the severity of the pathological changes corresponding with a progressive increase in the strength and amount of formalin injected. Likewise the severity of the symptoms following the injection of formalin into the stomach is by no means proportionate to the strength or amount of the injected chemical; nor is there a definite series of symptoms following the injection of a definite amount of formalin of a definite strength. The results obtained in the first three experiments in this series lead me to class formalin with that rare group of poisons which are capable of producing sudden death when swallowed.

Other things being equal, it might be expected that the rapidity with which death occurs after the injection of formalin into the stomach would be influenced by the following conditions: The amount or strength of the chemical; the time of retention after ingestion (absence of vomiting); the condition of the stomach; whether empty or not and the size of the animal experimented upon.

How small a rôle these factors play may be judged from the following: Although the rabbit of Exp. 2 was heavier and received only two-thirds the amount of formalin that was given to the rabbit of Exp. 4, the first died immediately after the ingestion of the chemical, while the second lived several days. Similarly, although the rabbits in Exp. 3 and 4 were identical in size, weight and age, and were injected under the same conditions, with the same amount of formalin, one died immediately while the other survived several days. In Exp. 6 the injection of 1 cc. of 10% formalin proved fatal in three and a half days. In Exp. 7 the injection of 3.5 cc. of formalin of the same

strength was followed by an attack of vomiting, and the animal lived nine days. It might be concluded that the animal's life was preserved because the toxic agent was vomited. Contrary to this conclusion are Exp. 8 and 9, for while the rabbit in Exp. 8 lived six days after the ingestion of 8 cc. of 5% formalin, the dog in Exp. 9 survived the administration of 17 cc. of the same strength by only forty hours, even though the animal began to vomit almost immediately after the ingestion of the chemical.

That the weight of the animal is not an important factor is evidenced by the fact that the dog, weighing nearly fifty times as much as the rabbit, was unable to withstand a proportionately smaller dose. The presence of food in the stomach does not seem to influence the toxic action of the chemical. In Exp. 1 and 2 post-mortem examination showed the stomachs filled with food yet both died immediately after the injection of the formalin. The rabbit of Exp. 4 was kept for several hours without food. It survived the injection of formalin for several days, yet its fellow, kept under exactly the same conditions, died immediately after the operation. In Exp. 9, on the other hand, the rapid death of the dog might be in part at least attributed to the ready absorption of the chemical by an empty stomach, for the animal had been kept without food for twelve hours.

That small amounts of formalin when taken into the stomach are capable of producing deleterious effects upon the animal economy is evidenced by the experiments of Annett and Grady.

Annett (9) noticed in kittens fed with milk containing formalin, loss of appetite and diarrhoea with noisy, gaseous motions, gaseous distension of the abdomen and roughening of the fur; in some instances there were emaciation and death. The younger animals were most susceptible, and showed, in contradistinction to the control kittens, little increase in weight with increase in age. F. F. Grady, of the health department of Chicago, has obtained similar results.

III. INTRAPERITONEAL INJECTION OF FORMALIN.

In this series of experiments twelve guinea pigs, two rabbits and five dogs were used. The injections were made through the abdominal wall, under antiseptic precautions, by means of a hypodermic syringe.

The symptoms following the injection of formalin into the peritoneum are fairly constant, and vary in intensity according to the strength and the amount of the injected chemical.

The anatomical findings after intraperitoneal formalin injections are quite similar. After the injection of 1-1000 formalin, macroscopic changes cannot be discovered. Sometimes there is slight vascular congestion. Marked vascular congestion with slight exudation follows the injection of 1% formalin. The intestines are usually contracted, especially at the points where they rub against the abdominal wall or against each other. When undiluted formalin is injected an abundant inflammatory exudate accumulates. Two days after the injection of 3.5 cc., I withdrew 160 cc. of a turbid, bloody fluid from the abdominal cavity of a dog. After coagulation the coagulum constituted 70% of its bulk. In the second dog 140 cc. of ascitic fluid were found post-mortem three days after the injection of 2.5 cc.; 90% of the total volume was formed by the clot after coagulation of the fluid. In the abdominal cavity of a third dog 50 cc. of an intensely bloody fluid were found eight days after the injection of 4 cc. of undiluted formalin. Histological examination of the ascitic fluid reveals desquamated epithelium, red blood corpuscles, large numbers of leucocytes and granular detritus.

The intestines after the injection of undiluted solutions are usually contracted. At the points of irritation are found ecchymoses varying in size. When these are of a mild grade they appear as delicate, subserous, reddish-brown or purple lines running parallel with the long axis of the gut. In the severer cases the entire thickness of the intestinal wall is soft and blood-soaked. Those parts of the intestinal tract which are protected by the omentum or by mutual contact may suffer but slightly in the destructive process, but the omentum may suffer severely. Slight ecchymoses are present in several of the ani-

mals even after the use of 1% solutions. When undiluted solutions are employed the fat columns of the omentum are seen as dark red, boggy masses of clotted blood. The parietal peritoneum shows changes similar to those described above. In one of my dogs extravasation of blood was present through the entire thickness of the abdominal wall. Fibrin is commonly found upon the omentum and mesentery, even when only dilute solutions (1-250) have been employed. In one of the dogs the pancreas was embedded in a fibrinous capsule. In another, fibrinous adhesions existed between all the pelvic viscera. The fibrin was always soft and oedematous.

The endothelial cells of the peritoneum after the injection of 1-1000 formalin, swell, show nuclear degeneration, and are loosened from the basement membrane. Sometimes single cells are found lying upon the connective tissue; at other times small plates of endothelial cells are found in process of desquamation. The muscularis is contracted and its nuclei compressed. In the outer muscular sheath the number of nuclei are diminished. Leucocytic infiltration, mainly polynuclear, has taken place into the serosa and between the layers of the muscularis; it is also found well-marked in the connective tissue of the submucosa. The mesentery is similarly infiltrated. Marked vascular congestion prevails.

Solutions of 1-250 formalin produce similar lesions.

All of the above changes, occurring with greater severity, are found after the injection of 1% formalin. The pathological findings range from an intense inflammation to total necrosis. The connective tissue elements stain intensely red with eosin and show a loss of nuclei. The mucosa is often represented by a mass of blue stained, necrotic material. The submucosa is crowded with polynuclear, and large and small mononuclear leucocytes; the mesentery shows similar changes. Marked vascular congestion and extravasation of blood are general. Abundant fibrinous exudation into the mesentery was found in some of the sections.

The following histological changes in the intestine were found after the injection of undiluted formalin into the peritoneal cavities of dogs:

The peritoneal lining cells are entirely lost in some places; in others, a few poorly stained cells still adhere to the subserosa. The subserosa is hyaline and stained red; its nuclei are decreased in number, misshapen and fragmented. The nuclei of the outer muscular tunic are lost and the muscular tissue is bright red, brownish or purple in color. The same is true but to a less marked extent of the inner muscular layer. The cells lying nearest the supporting connective tissue always suffer the most. The submucosa shows a fairly well-marked leucocytic infiltration. The mucosa suffers only slightly. Only in the dog which lived ten days after the injection could leucocytes in any considerable number be found in the subserosa and muscularis. The parietal peritoneum suffers changes similar to those affecting the visceral.

The most striking feature in these cases is the intense haemorrhage; in some of the sections examined this occupies the entire thickness of the intestinal wall except the mucosa. Blood cells or blood pigment are so abundant that the tissues into which the haemorrhage has occurred are unrecognizable. Well-preserved red blood corpuscles and granular pigment are often associated in the same section; and while one part of the intestinal wall shows naught but red cells other parts may show nothing but blood pigment. The mesentery is in all cases the seat of fibrinous inflammation, often accompanied by profuse haemorrhage.

The peri-pancreatic and interlobular connective tissue of the pancreas of the dog before alluded to is the seat of an intense fibrinous inflammation; the fibrin is delicately fibrillar, and mixed with leucocytes and granular necrotic material formed from the neighboring pancreatic parenchyma. The changes in this organ diminish in severity as its more central portions are reached; here the cells are ill contoured, granular, and show evidences of nuclear degeneration.

The peritoneal fat suffers markedly under the action of formalin. Frequently the fat cells are found filled with purplish, hyaline material. At other times, instead of the changes described above,

necrosis of fat cells has occurred and is accompanied by intense leucocytic infiltration. In the mesenteric fat, the deleterious action of the formalin most often manifests itself by the production of a fibrinous inflammation. Leucocytosis, karyolysis and karyorrhexis are often extreme.

The results of an experiment performed by Adler (10) agree well with my own. This investigator introduced several pith balls saturated with 4% formalin into the peritoneum of a rabbit. After twenty-four hours small ecchymoses were found on the surface of the colon where the pith cubes, surrounded by a soft, translucent, mucoid exudate had lodged. Microscopical examination of the cubes revealed polynuclear and large and small mononuclear leucocytes, plasma cells, "mastzellen," red blood corpuscles, tissue cells and epithelioid cells.

Borst (11) introduced fish bladders filled with blood into the peritoneal cavities of rabbits. When the bladders were sterilized in formalin and then washed in water, the usual cellular infiltration did not occur. This he believed to be due to a swelling of the cells in the fish bladder, or to a negative chemiotaxis exerted by the retained formalin.

The pancreatitis in one of my experiments is similar to the pancreatitis associated with fat necrosis which Flexner (12) produced in two dogs by injecting 2% formalin into the pancreatic ducts.

Experiments to determine the lethal dose of formalin introduced intraperitoneally were performed upon guinea pigs with the following result:

(a) Two guinea pigs died within two days after the injection of 1 cc. of a 1-1000 formalin solution for each 100 grm. of body weight.

(b) Five guinea pigs died within twenty-four hours after the injection of 2 cc. of 1-1000 formalin for each 100 grm. of body weight.

(c) One guinea pig, weighing 345 grm., died three days after the injection of 6 cc. of 1-1000 formalin.

(d) One guinea pig, weighing 306 grm., withstood the injection of 3 cc. of 1-1000 formalin; at the end of four weeks the animal weighed

400 grm., and withstood the injection of 8 cc. of formalin of the same strength.

(e) Four guinea pigs were killed by 2 cc. of 1% formalin in periods ranging from six to twelve hours.

IV. INJECTION OF FORMALIN INTO THE LUNG.

In these experiments slow injection through the chest wall was resorted to.

Exp. 1.—Guinea pig, wt. 405 grm. Injected 1 cc. of 1-1000 formalin into right lung.

Exp. 2.—Guinea pig, wt. 372 grm. Injected 2 cc. of 1-1000 formalin.

Both animals remained apparently well. The first was killed on the sixth and the other on the second day after the injection.

On post-mortem examination the lung on the injected side is not as perfectly collapsed as that of the other side, and on cross-section the lung tissue feels slightly granular.

Exp. 3.—Terrier. Injected 5 cc. of 10% formalin into right lung. After two days, during which the animal coughed from time to time, the dog was killed.

Post-mortem shows only the right middle lobe to be affected. This does not collapse, is firm, and does not crepitate; pieces of it sink in water. The cut section is brownish-red and from it drips a light, straw-colored fluid. In the center of the lobe is found a sharply defined red area the size of a dime. The bronchi contain thin mucus.

Exp. 4.—Terrier. Injected 3 cc. undiluted formalin into right lung. Death occurred in 18 hours.

On post-mortem examination the left lung is pink in color and crepitates throughout. The right lung is dark red, firm, non-crepitant, and sinks in water: from the cut section drips a bloody fluid. In the center of the lower lobe is found a cavity 2 cm. in diameter filled with a bloody, necrotic material.

Exp. 5.—Large cat. Injected 3 cc. of undiluted formalin into the right lower lobe. The animal died immediately.

On post-mortem examination a brownish-black solid area, the size of a small cherry, is found in the right lower lobe. The bronchi of the corresponding side contain bloody froth.

Histological examination of the lungs of the animals in the first two experiments reveals pneumonia on the right side. There is a

slight leucocytic infiltration of the alveolar walls, together with capillary congestion. Scattered throughout the lung substance, but especially common about the larger blood-vessels and the bronchi, are circumscribed areas of leucocytic infiltration. Here the alveoli are filled with eosinophiles, mononuclear and polynuclear leucocytes. Sometimes red blood corpuscles are found, but it is possible that their presence is due to the trauma consequent upon the hypodermic puncture. There is desquamation of the lining cells in some of the bronchi.

In Exp. 3 the pneumonia is much more severe. The dark area described in the anatomical findings is made up of red blood corpuscles, blood pigment and necrotic lung tissue. About this necrotic focus is a zone of intense leucocytic infiltration. Leucocytosis of a less severe type extends throughout the tissue of the affected lobe. About the blood-vessels and bronchi denser areas of infiltration are noted. The leucocytes are mainly of the polynuclear variety. Eosinophile and mononuclear leucocytes are comparatively rare. All the alveoli are filled with a homogeneous, red-stained exudate, mixed with leucocytes, strands of fibrin and desquamated alveolar epithelial cells. There is intense capillary congestion and the large vessels are also well filled. Extravasation of blood has occurred in many localities. The bronchi contain leucocytes, red blood corpuscles and necrotic material. Total loss of bronchial epithelium is a common feature of the bronchitis. The walls of the bronchi are oedematous and infiltrated with leucocytes.

The lungs in Exp. 4 show practically the same characteristics as those in the experiment just described. There is greater extravasation of blood and in places, actual breaks in the vessel walls can be made out.

In Exp. 5 the only discoverable change is a most intense capillary congestion. Except for an occasional red blood corpuscle the alveoli are empty.

V. SUBCUTANEOUS INJECTION OF FORMALIN.

Formalin, varying in strength from 0.1% to 10%, was injected under the skin of the hind leg, or under the skin over the ribs of guinea pigs and rabbits.

The histological findings were, in all cases, quite similar. They varied in severity and extent, with the strength and with the amount of injected formalin. The characteristic lesion is an intense exudation into the subcutaneous tissues. There is found underlying the skin a yellowish, jelly-like mass, from which when cut across drips a large amount of light straw-colored fluid. Such an exudate is most abundant eighteen hours after the injection of formalin, and may then be 3 or 4 cm. in thickness. Absorption of the exudate usually occurs in a few days, but may be markedly delayed when the formalin is not diluted. A marked proliferation of the connective tissue is concomitant with the absorption of the exudate.

The epithelial covering shows no changes. The exudate is stained red, and mixed with cellular debris, isolated connective tissue cells and leucocytes. All forms of the latter are present, but polynuclear cells, especially the eosinophiles, predominate in the specimens obtained soon after the injection. The number of connective tissue cells (formative cells) varies with the length of time that has elapsed since the injection, and with the strength of the formalin. Active proliferation of the existing connective tissue can be found on the second or third day after injection. When strong solutions of formalin have been used, proliferation is at first delayed, but once started, becomes excessive. A delicately fibrillar fibrin, differing in amount in the various experiments, is found scattered through the exudate. The blood-vessels are enormously congested.

VI. THE EFFECT OF FORMALIN UPON THE MUSCLES.

After the injection of formalin varying in strength from 0.1% to 100% into the leg muscles of dogs and rabbits, it is usually found at autopsy that the chemical has acted upon a circumscribed area of tissue. When strong solutions have been employed, the affected

muscle is brown in color, hard and dry, cutting much like a piece of dried bread. Exudation into the fascia usually takes place and soft boggy lines run through the dry areas. When dilute solutions are used the exudation is more severe, and the area of muscular tissue acted upon by the chemical is dark red and soft and from it drips a slightly bloody fluid. In one dog, I found within the muscular tissue fifteen days after the injection of 4 cc. of 10% formalin, a cyst containing straw-colored serum and a mass of clotted blood. The walls of the cyst had been formed by active proliferation of the connective tissue of the fascia.

Histologically, we have to deal with a myositis of varying severity. Between the muscular fibers dense aggregations of leucocytes are found. In specimens examined twenty-four hours after the injection of the formalin, infiltration is mainly of the polynuclear variety; in older specimens the mononuclears become very numerous. A homogeneous red-stained exudate often mixed with fibrin is found in the intermuscular septa. When a few days have elapsed since the injection of formalin, evidences of connective tissue proliferation manifest themselves. In the course of two or three weeks this becomes excessive. The muscular fibers are sometimes swollen, sometimes shrunken and wavy; they are hyaline and stain bright red. Total loss of nuclei and muscular striation is common. In many places segmentation of the fibers has occurred. Often, especially when strong solutions have been employed, the muscular fibers are shrunken and stain blue with haematoxylin; at other times the musculature stains a uniform deep purple. In these localities no leucocytes can be found. Any or all of the above conditions may be present in a single field of the microscope.

It is not necessary that the formalin be injected into the tissues in order that all the above changes may occur. Changes identical with those described occur in the musculature of the eyelids when formalin is dropped into the eyes, in the abdominal wall after intraperitoneal injections, or in the skeletal muscles after the chemical has been injected subcutaneously.

When formalin comes in contact with non-striated muscular tissue, changes similar to those observed in voluntary muscles result. In the intestinal wall, for example, after the injection of formalin into the peritoneum or into the stomach, the muscle cells are contracted, stain red with eosin or, if the destructive action has been more marked, purple with haematoxylin. A loss of nuclei is very common, and slight leucocytic infiltration is occasionally present.

VII. THE EFFECT OF FORMALIN AND FORMALDEHYDE UPON THE EYE.

Lacrymation and congestion of the conjunctival vessels are present in the eyes of all animals exposed to the vapors of formaldehyde. The intensity of these symptoms varies only slightly with the length of exposure, and seems to depend more upon the concentration of the gas to which the animals are exposed. The symptoms usually subside within twenty-four hours. Histological examination a few hours after exposure to the gas shows only vascular congestion.

The slight cloudiness and pupillary contraction which follow very soon after the injection of formalin, up to the strength of 1-500, into the anterior chamber of the eye, always pass off in a few hours. When a drop or two of a 5% solution are injected definite changes can be found after twenty-four hours. The anterior chamber becomes filled with delicately fibrillar, densely matted fibrin, with which polymorphonuclear leucocytes are mixed. These are most numerous in the angle between the iris and the cornea. The lining cells of the anterior chamber are swollen and often desquamated.

In three animals in which a few drops of 10% formalin were dropped into the eyes no changes except vascular congestion of the eyeball and eyelids resulted. For several hours after the introduction of the formalin there was intense lacrymation.

A single drop of undiluted formalin is sufficient to injure an eye permanently. The changes which result are generally as follows: Immediately after the introduction of the formalin there is lacrymation, blepharospasm and contraction of the pupil. After twenty-four hours the pupil is contracted to almost pin-hole size, and refuses

to dilate after the use of atropine. Intense oedema of the eyelids develops and prevents their closure; the cornea becomes dry and opaque. At the end of three days the lids become less oedematous and their margins may become agglutinated through fibrinous exudate.

The conjunctiva and cornea become infiltrated with leucocytes. In the angle between the visceral and parietal conjunctiva large collections of leucocytes are found; twelve or eighteen hours after formalin has been dropped into the eye the cornea is oedematous and its corpuscles show evidences of proliferation. About the canal of Schlemm there is intense infiltration with polynuclear leucocytes. The anterior chamber of the eye is filled with exudate stained homogeneously red with eosin and mixed with delicately fibrillar fibrin, and large numbers of leucocytes. The intensity of the process is subject to great variation.

As in the eye, the changes in the eyelids do not always give a definite reaction to a definite amount of injected formalin. When the reaction is but slight, there may be only well-marked leucocytic infiltration of the mucous lining of the eyelids; the muscular tissue may assume a brownish color after staining with haematoxylin and eosin; sometimes myositis is present. In very severe cases the mucous membrane is covered by a layer of fibrin, the blood-vessels are markedly congested, the tissues are oedematous, and between the necrotic cells are found masses of fibrin.

In addition to the local effects following the injection of formalin, pathological changes occur in the parenchymatous organs in consequence of the absorption of the chemical from the point of injection. These lesions will now be considered.

VIII. CHANGES IN THE LIVER.

Among the general changes produced by formalin in whatever way it is introduced into the body are those occurring in the liver. These consist essentially of cloudy swelling, varying in intensity associated with vacuolation of protoplasm and destruction of the nuclei; ultimately total destruction of cells may occur.

Changes in the liver may be noted after injections into the peritoneum of even dilute solutions. In guinea pigs after the injection of from 4 to 8 cc. of a 1-1000 solution, these changes are of a mild form; the liver cells are swollen, their outlines obscured and the protoplasm granular; the nuclei show no changes. After the injection of 1% solutions the changes are more severe. The columnar arrangement of the cells is partially lost and definite boundaries between the rows of liver cells cannot be distinguished. The cells are more swollen, the granulation is coarser than normal, and the cell outlines are entirely obscured; the nuclei in a few cases are somewhat crenated. The cells about the central veins stain more lightly than those at the periphery of the lobules. Accompanying these changes is a general vascular congestion; occasionally a leucocyte is found in the tissue.

Intraperitoneal injection of undiluted formalin into dogs results in the production of a diffuse cloudy swelling of the liver; the cells are swollen, the outlines lost and the protoplasm is granular. The nuclei are crenated and of bizarre shapes. Between the rows of liver cells are found a few polynuclear and mononuclear leucocytes. The interstitial tissue contains many nuclei and the blood-vessels are congested. These changes are noted from the second to the fourth day after the injection of formalin.

Subcutaneous injections of formalin are capable of producing only slight cloudy swelling of the hepatic cells. Severe changes cannot be found even after the use of strong solutions.

The most severe changes in the liver were noted in those experiments in which formalin was injected into the lungs or into the stomachs of animals, and after the inhalation of the vapors of formaldehyde. The changes under these conditions are very uniform; the columnar arrangement of the liver cells is almost entirely lost and the cells are enormously swollen, some of them having attained fully three or four times the size of the normal cell. The cellular outlines can rarely be distinguished and the protoplasm is greatly vacuolated and granular. The appearance of fatty degenera-

tion is produced, but the irregular contour of the vacuoles speaks against this view. The nuclei are somewhat swollen but have preserved their smooth contour in many cases; at other times the nuclei are crenated or bizarre in shape. Between the rows of liver cells are many polynuclear and occasionally mononuclear leucocytes. Figures simulating karyokinesis are found, but the exact nature of these I could not determine. It is possible that they represent broken down nuclei or polymorphonuclear leucocytes. An increase in the number of nuclei in the interstitial connective tissue is due either to cell proliferation or to infiltration with mononuclear leucocytes. The blood-vessels are filled with blood. The greatest leucocytic infiltration occurred in a dog into the lungs of which 10% formalin was injected.

A marked contraction of the hepatic cells associated with vacuolation, instead of the ordinarily observed swelling, is found in some of the specimens. In no case was I able to find an increased number of leucocytes in the liver sooner than forty hours after the injection of the formalin. In four of the animals which had inhaled formaldehyde I found areas of focal necrosis in the liver. Such foci are sometimes small and six or twelve liver cells have undergone destruction (Fig. 4); at other times areas as large as half a liver lobule are destroyed. Mixed with the granular debris and the shrunken and broken down remains of liver cells are polynuclear and mononuclear leucocytes. Eosinophiles are rarely found. That regeneration of the cells occurs is indicated by the fact that no histological changes can be discovered in the livers of animals which survive for two or three weeks the intraperitoneal injection of dilute formalin.

Hensen (13) has produced parenchymatous changes in the liver by injecting 1.5 cc. of from 0.5 to 4% formalin into the gall-bladders of cats. The changes varied from slight cloudy swelling to total necrosis of the liver cells. In all cases only a slight inflammatory reaction occurred, and evidences of regeneration were plentiful. My own observations agree well with those of Hensen, and it is of interest

to note that formalin, no matter in what way introduced into the body can produce lesions in the liver similar to those resulting from the injection of the chemical directly into the organ.

IX. CHANGES IN THE KIDNEYS.

Changes in the kidneys are constantly present after the injection of formalin. Definite changes are noted in the kidneys within six or eight hours after the injection of from 3 to 6 cc. of 1-1000 formalin into the peritoneal cavities of guinea pigs. Macroscopically the kidneys are firm, swollen, dark red and bloody. Microscopically, the glomeruli show an indistinct structure. The sepearate cells cannot be distinguished from each other; the nuclei are polymorphous or crenated, and often show figures suggesting karyokinesis. The uriniferous tubules show a variety of changes; for the most part the cells lining the convoluted tubules are swollen, granular, and stained red with eosin, while often a faintly reticular structure can be made out. The cells are swollen to the extent of occluding completely the lumina of the tubules, and the cellular outlines are obscured. The nuclei of the cells are pale and swollen, and some have entirely disappeared. In some areas in the kidney the epithelial lining of the tubules has been lost so that nothing but empty holes remain; sometimes these holes contain a small amount of granular material, at other times, desquamated cells with fragmented nuclei. Often the entire epithelial lining is contracted and found lying free in its connective tissue casing. Some of the tubules are filled by a homogeneous, bluish staining mucus-like material. The changes in the straight tubules are similar to those in the convoluted tubules, the cells being swollen and granular. Often the arrangement of the cells upon the basement membrane is disturbed and sometimes total cellular necrosis has occurred, only the empty connective tissue tubules remaining.

In two guinea pigs, one dying eight, the other twelve hours after the intraperitoneal injection of 4 cc. of a 1% solution of formalin, all of the changes described above are found. No definite

structure can be made out in the glomeruli and intense vacuolation of the cells has occurred. In the glomeruli and in the tubules an occasional polymorphonuclear or mononuclear leucocyte can be seen and blue hyaline material is found in some of the tubules.

The administration of formalin by the stomach or its injection into the lungs produces the same changes in the tubules of the kidney as those just described. The same result follows intraperitoneal injections of undiluted formalin into dogs. The glomerular changes however, are of a severe type. In one case after the injection of 3.5 cc. of undiluted formalin some of the glomeruli are entirely lost and in their places a small amount of poorly stained granular material is found. In another dog there are found only empty tubules containing red vacuolated bodies, probably the remains of necrosed cells.

In the animals exposed to the fumes of formaldehyde the cells of the uriniferous tubules are the seat of intense vacuolation. Further, the changes already described are found and some of the glomeruli are entirely destroyed. In three of the animals exposed to the fumes of formaldehyde areas of focal necrosis were found in the kidneys. Such foci are usually of small size, and cover an area corresponding to the cross-section of two or three tubules. Mixed with the granular debris and the broken down nuclei of the destroyed epithelium are found mononuclear and polynuclear leucocytes.

X. CHANGES IN THE LUNGS AFTER ABSORPTION OF FORMALIN.

Pneumonia and bronchitis, with or without haemorrhage, are found in the lungs of all animals after the injection of formalin. These changes are of about the same type in all the animals, and do not vary in severity with variations in the strength and the amount of the injected chemical.

The bronchi contain desquamated bronchial and alveolar epithelium, together with polynuclear leucocytes. The lining cells of the bronchi are swollen and granular, and in places partially desquamated. Many of the alveoli contain a finely granular or hyaline

material (oedema) with which are mixed desquamated alveolar epithelial cells and leucocytes. The epithelial cells of the alveoli are swollen and granular, and their nuclei are pale and misshapen. Many of these cells are in process of desquamation. The blood-vessels are congested and contain a large number of leucocytes.

XI. CHANGES IN OTHER ORGANS.

Except for an apparent increase in the number of polynuclear leucocytes, I was unable to find changes in the spleen of any of the animals experimented upon. There was always an abundance of blood pigment present and many of the leucocytes were filled with it. In the heart I found no definite changes. The suprarenals were unchanged.

A distinct eosinophilia was found in the bladders of two dogs. In addition the epithelial cells of the bladder had undergone well-marked necrosis (the cells were swollen and granular, with crenated and broken down nuclei) and desquamation. To what extent these changes were pathological, I am unable to say.

In the spinal cord and brain of four animals examined by Nissl's method, the ganglion cells showed chromatolysis, peripheral and central migration of the Nissl bodies, and eccentric location of the nuclei.

XII. CHRONIC FORMALIN POISONING.

Four animals were subjected to chronic formalin poisoning, receiving a series of intraperitoneal injections of dilute formalin. The results were as follows:

Exp. 1.—Rabbit, wt. 920 grm. Injected 6 cc. of 1-2000 formalin on four successive days. The animal seemed slightly uncomfortable but otherwise well. The rabbit was killed ten days later when no macroscopic changes could be found in the organs.

Exp. 2.—Guinea pig, wt. 765 grm. Injected 4 cc. of 1-2000 formalin on four successive days. The animal lost 27 grm. in weight. Twelve days later the pig had regained its former weight and formalin injec-

tions were recommenced. Injections of 4 cc. of the chemical, beginning with 1-2000 and gradually increasing in concentration, were made every second day. No symptoms except a slight restlessness followed the injections. The animal succumbed after the ninth injection—4 cc. of a 1-625 formalin solution. At death the animal weighed 702 grm. Time of experiment 42 days.

Exp. 3.—Terrier, wt. 25 kilo. The experiment extended over a period of 38 days. The following injections were made, one day at least intervening between the consecutive injections:

Ten injections of 8 cc. of 1-1000 formalin; one injection of 16 cc. of 1-1000 formalin; three injections of 24 cc. of 1-1000 formalin; one injection of 32 cc. of 1-1000 formalin; one injection of 40 cc. and one of 50 cc. of 1-500 formalin. The dog was killed with chloroform.

Exp. 4.—Rabbit, wt. 1675 grm. The animal was observed for 106 days. The following injections were made, one day at least intervening between injections:

Twelve injections of 6 cc. of 1-1000 formalin; two injections of 8 cc. of 1-1000 formalin; one injection of 12 cc. and one injection of 20 cc. of 1-1000 formalin.

I now began to increase slowly the concentration of the chemical, as well as to increase the amount injected. After increasing the strength of the formalin I would at first decrease the amount injected so as to make the transition with as little shock as possible to the animal. In 61 days the amount of the chemical injected was raised from 16 cc. of 1-500 to 40 cc. of 1-150 formalin. After the ninth injection of 1-1000 formalin the animal had lost 75 grm.; thereafter the animal gained in weight and weighed, when killed, 1920 grm. For several days the animal weighed 2026 grm. After a series of injections the rabbit would lose weight; the weight would be regained when the injections were stopped. It was also noticed that an increase in the amount of formalin injected was followed by a corresponding increase in the loss of weight.

On post-mortem examination no macroscopic changes of a type much severer than those ordinarily observed could be found. The kidneys and liver of the dog in Exp. 3 were yellowish. In the rabbit of the last experiment the intestines had a distinctly granular feel, and the mesentery was firmer and thicker than normal. The capsules of the kidneys were also thickened.

Histological examination of the viscera gave the following result: In the first three experiments there is fibrino-haemorrhagic peritonitis such as I have already described. In the third experiment organization of the exudate is well advanced, while in the last experiment there is enormous proliferation of the connective tissue. The mesentery is made up almost entirely of embryonal connective tissue, and the serosa of the intestines and abdominal wall is very markedly thickened. Enormous proliferation of connective tissue has also occurred in the omentum. In places areas of fibrin mixed with red blood corpuscles are undergoing organization. A most striking infiltration with eosinophile leucocytes is present in the mesentery, omentum, serosa and submucosa of the intestines, and in the abdominal wall. Other polynuclear and mononuclear leucocytes are present but they are comparatively few in number. About the smaller blood-vessels and irregularly scattered through the young connective tissue are small, densely-crowded aggregations of lymphocytes.

The capsules of the kidneys, spleen and pancreas of the rabbit in the last experiment are thickened, show active proliferation of connective tissue, and are infiltrated with eosinophile and other forms of leucocytes. These changes, it seems to me, are due to the formalin which came in contact with these organs. It is probably the result of this proliferation of connective tissue and thickening of the serosa that the animals are able to bear without apparent suffering the injection of large quantities of the stronger solutions employed in the later experiments.

Severe cloudy swelling is present in the livers of the animals used in the last three experiments. In the dog there was marked fatty degeneration. Areas of focal necrosis are found in the liver of the guinea pig. In the guinea pig, and in the rabbit, more markedly in the latter, the interlobular connective tissue is infiltrated with small mononuclear leucocytes. Slight leucocytosis is present in the livers of all the animals except the first.

The kidneys are the seat of diffuse cloudy swelling. Fatty degeneration is marked in the kidneys of the dog (Exp. 3). The connec-

tive tissue in the kidneys of the last three animals is infiltrated with small mononuclear leucocytes, but other forms are rarely found. Areas of focal necrosis are found in the kidneys of the guinea pig. In the dog extensive areas in which kidney substance has disappeared are infiltrated with leucocytes of the mononuclear variety (Fig. 5). Many plasma cells are present.

The lungs of the four animals show the slight bronchitis and pneumonia usually present after the administration of formalin. Their spleens contain a large amount of blood and yellow pigment and in the last experiment a large number of eosinophiles.

I take pleasure in expressing my thanks to Dr. Hektoen for suggesting the subject of this paper to me; and for continued interest and guidance during its preparation.

SUMMARY.

The results of this investigation may be summarized as follows:

1. The inhalation of formaldehyde gas in even small quantities is followed by bronchitis and pneumonia. Pneumonia is due to the inhalation of the gas and not to secondary infection.

2. Formalin belongs to that rare group of poisons which are capable of producing death suddenly when swallowed.

3. The introduction of formalin into the stomach is followed by the production of a gastritis which varies greatly in character. The duodenum and upper jejunum may also be involved in the inflammatory process.

4. Intraperitoneal injections of formalin cause peritonitis of a fibrino-haemorrhagic character. A definite reaction is obtained when very dilute formalin (1-1000) is employed. In the peritoneal cavity formalin exercises a destructive action upon all organs (pancreas, liver, peritoneal fat, Fallopian tubes, etc.) with which it comes in contact and causes inflammation in these organs.

5. The lethal dose of formalin when injected intraperitoneally into guinea pigs is approximately 2 cc. of 1-1000 formalin for each 100 grm. of body weight.

6. The injection of formalin into the lungs is followed by pneumonia and bronchitis.

7. The inflammation which follows subcutaneous injections of formalin is characterized by intense exudation.

8. The injection of formalin into the muscles produces myositis.

9. The injection of formalin into the anterior chamber of the eye causes the accumulation of an exudate containing leucocytes and fibrin. When formalin is dropped into the conjunctival sac iritis follows and may be severe enough to destroy the eye.

10. Formalin in whatever way introduced into the body is absorbed, and is then capable of producing lesions in the parenchymatous organs.

11. Changes in the liver after absorption of formalin consist of mild or severe grade of cloudy swelling accompanied by vacuolation of the protoplasm, changes in the nuclei and leucocytic infiltration. Focal necrosis may result. Similar changes follow the inhalation of formaldehyde.

12. The injection of formalin or the inhalation of the vapors of formaldehyde produces cloudy swelling of the parenchyma of the kidney. Focal necrosis may result.

13. Pneumonia and bronchitis are found in all animals after the injection of formalin.

14. The leucocytic infiltration which follows the introduction of formalin into an organ has these general characteristics: The eosinophiles are the first leucocytes to appear; these are followed by the other polynuclear leucocytes; last appear the large and small mononuclear leucocytes. Similar phenomena occur in the trachea, bronchi and lungs of animals subjected to formaldehyde inhalations.

15. Formalin is, directly or indirectly, chemiotactic for leucocytes. The tissues which are not infiltrated with leucocytes after the injection of formalin are those which have been so injured by the chemical that an inflammatory reaction is impossible.

16. Animals subjected to chronic poisoning with formalin administered by intraperitoneal injection develop fibrinous peritonitis,

associated with marked eosinophilia. The changes in the kidneys and liver consist of cloudy swelling, fatty degeneration, focal necrosis and leucocytic infiltration.

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EXPLANATION OF PLATES.

Fig. 1. Eosinophilic pneumonia in a guinea pig exposed for one and a half hours to formaldehyde. (Sec. I, Exp. 1.)

Fig. 2. Pneumonia at a later stage, in a rat exposed for three and a half hours to formaldehyde. (Sec. I, Exp. 2.)

Fig. 3. Eosinophilic tracheitis in a guinea pig exposed to formaldehyde inhalation for three and a half hours. (Sec. I, Exp. 2.)

Fig. 4. Focal necrosis in the liver in a guinea pig killed forty-two hours after exposure for five hours to the vapors of formaldehyde. (Sec. I, Exp. 4.)

Fig. 5. Extensive leucocytic infiltration found in the kidney of a dog subjected to chronic poisoning with formalin. (Sec. XII, Exp. 3.)

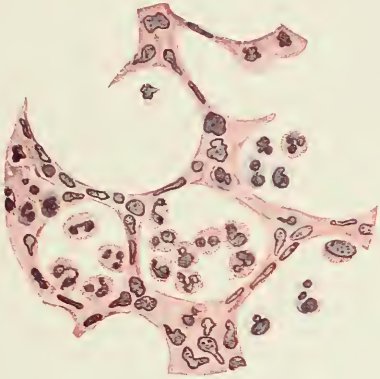


FIG. 1.

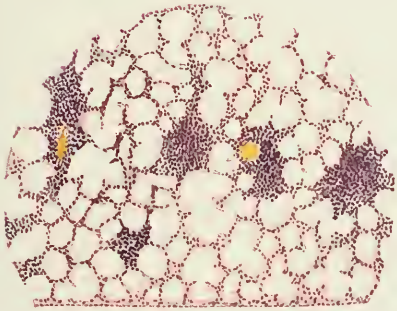


FIG. 2.

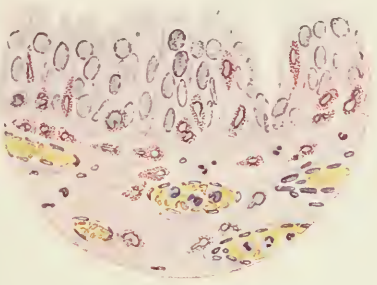


FIG. 3.



FIG. 4.



FIG. 5.

BACILLUS MORTIFERUS (NOV. SPEC.).

By NORMAN MAC LEOD HARRIS, M. B. (Tor.)

(From the Pathological Laboratory of the Johns Hopkins University and Hospital.)

It is proposed in this paper to describe a hitherto unknown bacillus which is pathogenic for man and animals.[1]

Source.—From an abscess in the liver of a person who died and came to autopsy in the Johns Hopkins Hospital on October 13, 1900.

Clinical Report.—For the following clinical notes I am much indebted to the kindness of Professor William S. Halsted, under whose care the patient was admitted.

J. B., æt. 44. Admitted to the Johns Hopkins Hospital October 6, 1900. The family and past histories are unimportant.

Present Illness.—Note of October 6: The patient was admitted with a history of headache of four days' duration. Three days before admission nausea began, accompanied with vomiting, which continued until last night. The patient thinks the vomitus had a faecal odor and was dark yellow in color. He was unable to take food until 24 hours after vomiting. Abdominal pain began two days ago and was constant, not paroxysmal, throughout the whole right side. He places his hand to the right of the umbilicus and to the upper right quadrant of the abdomen. The bowels have moved daily three or four times and contain no blood. There was a free movement last night with flatus. He has had chills, one at 8 P. M. last evening and one at 2 A. M. this morning, followed by profuse sweating. The patient never had previous similar chills, and no trouble of the kind is known in his neighborhood. He drinks hydrant water. Temperature 103° F. Micturition normal.

Physical Examination.—Patient walks erect. He is large, well developed, of good color, skin moist, no jaundice, conjunctivae clear. Tongue slightly coated. He complains of intense pain when head is touched. There is no cervical rigidity. Pupils react equally to light. He is rather

[1] Presented at the second annual meeting of the Society of American Bacteriologists, in Baltimore, Md., Dec. 27 and 28, 1900, and at a meeting of the Medical Society of the Johns Hopkins Hospital, on Jan. 7, 1901. A preliminary report was published in the *Bulletin of the Johns Hopkins Hospital*, 1901, xii, 216; likewise in the *Centralb. f. Bakt.*, 1901, xxix, 447.

excitable. Abdomen: symmetrical respiratory movements are present throughout the whole abdomen. Upon deep inspiration he complains of pain in the upper right quadrant. The urinary bladder is not distended. There is no dulness in the flanks. Liver dulness begins at the 6th rib in the mammillary line of the abdomen. Midway between the median line and the umbilicus, dulness proceeds 8 cm. below the costal margin. On palpation the left side of the abdomen is soft and there is no tenderness and no rigidity of the rectus muscle in its lower half. Above the umbilicus, however, the rectus is rigid and pressure causes the patient to wince. The lower part of the right rectus is also relaxed, but there is slight tenderness on deep pressure. In the right iliac fossa, as throughout the whole right abdomen, there is some tenderness with resistance on deep pressure. There is no muscular spasm in this region. As the upper right quadrant is approached, tenderness becomes more marked, rigidity greater, and just above the umbilicus pressure on the rectus causes the patient to cry out. Over the dull area there is definite muscle spasm and great rigidity of the muscles, and this spasm likewise extends to the rectus above the umbilicus and to the other abdominal muscles around the margin of the dull area. No definite mass can be felt beneath the area of muscle spasm. Tenderness extends into the right flank, but the percussion note is resonant. No tenderness exists in either lumbar region.

There are no signs of hernia. The genitalia are negative. Rectal examination shows no tenderness and no mass. Rectal temperature 104° F. Pulse 100 per minute. Respiration 34 per minute. Leucocytes 36,600.

Evening note, 9 P. M.: Patient has complained of pain in right side of the abdomen throughout the afternoon and says that he feels worse than upon admission. Temperature by mouth at 8 P. M., 105.8° F.; by rectum at present, 105.4° F. Pulse between 100-120 per minute. Leucocytes 7000. Patient looks worse. Face is flushed; skin hot; no nausea and no vomiting.

Abdomen.—Respiratory movements present. There is slight distension. Tenderness has increased throughout the right side and the area of muscle spasm has extended further down the rectus and also further downward and outward in the right abdomen. There is, however, no general tenderness or spasm. The area of dulness seems to have decreased (probably due to increased distension).

October 7. Patient's temperature is still high, between 103°-105° F. There is less pain and tenderness in the abdomen. Leucocytes 8000.

October 8. The patient seems more comfortable to-day. Leucocytes at 11 A. M., 8000; at 10 P. M., 16,000. Pulse 98-100. Temperature at 2 A. M., 100.5°; at 10 A. M., 103.5°. Gruber-Widal test, negative.

October 9. Abdomen looks normal. Peliomata striking. There is no special tenderness present. Abdominal walls are held tense, especially over region of the gall-bladder, so that it is difficult to palpate.

Leucocytes at 11 A. M., 20,600.

Leucocytes at 3 P. M. (before operation), 19,000.

Leucocytes at 5 P. M. (immediately after operation), 22,000.

Note by Dr. Mitchell at 3 P. M.: During the last 24 hours there has been a marked change in the patient's mental condition, more or less delirium having developed accompanied with mental dulness. He responds intelligently to questions, but seems much more stupid than on admission. Dr. Halsted saw the patient at 1 P. M. and advised operation. Percussion by Dr. Halsted showed the area of dulness to be exactly as it was upon admission. Otherwise there is little change since yesterday. The abdominal condition has cleared up markedly since admission, muscle spasm having disappeared, leaving only the dulness and a lessened degree of tenderness. The bowels have moved freely and unaccompanied by vomiting. Temperature has been irregular, but high.

Operation by Dr. Mitchell.—Exploratory laparotomy under Schleich's solution plus morphia gr. $\frac{1}{6}$. Demonstration of multiple abscesses in the liver. General anaesthesia. Rupture of abscesses on under surface of liver near the gall-bladder. Packing off with bismuth gauze. Appendix negative. Four abscesses opened and cultures taken. Iodoform gauze drain inserted. Partial closure with silver wire.

Under Schleich's solution an exploratory incision was made through the right rectus muscle. A little clear fluid was seen in the peritoneal cavity and cultures were made. General anaesthesia was induced for exploration, as great pain was caused by touching the liver or parietal peritoneum.

The area of dulness was found to be due to a large right lobe of the liver. The left lobe projected beyond the median line. The liver was large, its edges thin. The area of muscle spasm was indicated by an injected haemorrhagic condition of the parietal peritoneum. On the surface of the liver several abscesses were seen, measuring 1-3 cm. in diameter. One of these on the left lobe was fluctuant, its surface bright yellow. About it was some fibrin as in fibrinous peritonitis. In the right lobe were several other abscesses.

The liver was packed off and the abdominal cavity explored. The

appendix seemed normal. The intestines were pale and distended. There was nothing to suggest primary infection. In exploring the liver ducts, pus was seen escaping from the lower surface of the liver near the cystic duct, and was found to flow from an abscess on the under surface of the liver which had probably ruptured during manipulation. There were about two ounces of thin, foul, bright-yellow pus which was followed by yellow necrotic-looking material. The abscess cavity was packed with a piece of iodoform gauze to protect the intestines. The gall-bladder was not distended and contained no stones. Its ducts were not explored further but were probably patent, as the gall-bladder seemed to empty itself during manipulation.

Bismuth gauze packing was now placed around the lower surface of the liver and peritoneum above. The abscesses in the left lobe were incised and from three drams to one ounce of thin, foul, pale-yellow pus with flocculent necrotic masses evacuated from each. A clamp could be passed into the substance of the liver to a distance of 5-10 cm. The liver seemed as a whole very soft. A strip of iodoform gauze was placed in each abscess. No other abscesses were seen, but the finger could detect uneven places on the surface of the liver beneath the ribs.

The abdomen was partially closed with mattress sutures of silver wire in the muscle, and with subcutaneous silver wire sutures.

October 10. Patient has been fairly comfortable since operation. Temperature has ranged between 99° and 105°. This afternoon patient had a chill and temperature rose from 103.6° to 105°. Pulse 96 per minute. General condition about the same as before operation. Leucocytes at 11 A. M., 24,000; at 4 P. M., 8600.

October 11. 8.30 A. M., leucocytes 16,800; 5.30 P. M., leucocytes 21,000.

Patient was redressed; the dressings were soaked through with a sanguino-purulent discharge. Temperature has ranged from 102-104.6°. He has been drowsy, at times very restless and anxious to go home. Conjunctivae still yellow, and the skin over the whole body has a slight yellow tinge. Patient has constant thirst. He talks rationally at times, but more often is decidedly irrational.

October 12 and 13. Patient has grown weaker and this morning (October 13) can hardly be aroused. Temperature 102.8°. Pulse 120 and very weak. Respirations 52 per minute. Extremities are cold and cyanosed. Dyspnoea is quite marked. General condition much worse. The jaundice has increased. Tremor still very marked. Death at 3.30 P. M.

Autopsy No. 1608.—Pathological Report.—The autopsy was performed by Dr. Eugene Opie on October 13, 1900, at 8.30 P. M., the body having been dead five hours. An abstract only is given here.

Anatomical Diagnosis.—Multiple abscesses of liver, lungs and spleen. Jaundice. Localized fibrinous pleurisy. Enlargement and hyperaemia of portal and retroperitoneal lymph-glands. Laparotomy wound below right costal margin, packed with gauze and extending to the surface of the liver. Acute interstitial nephritis.

The body is that of a large, muscular man, 172 cm. in length. Rigor mortis is present. The skin of the entire body has a fairly deep yellow hue. Below the right costal margin is a linear incision longitudinally disposed into which pass a large number of gauze strips, separated from the edges of the wound by rubber protective. Subcutaneous fat is abundant.

Peritoneal Cavity.—It contains no excess of fluid. The general peritoneal surface is smooth. The gauze drains referred to pass, a few over the upper surface of the liver a short distance, the majority to the lower surface between the liver and the gall-bladder and colon with its mesentery, and are firmly adherent to the surfaces with which they are in contact. These tissue surfaces are dull and in places of a black color. Strips of gauze enter three small cavities in the liver.

The large intestine is considerably distended. The appendix lying in the right iliac fossa is free of any adhesions.

Thorax.—The pleural cavities contain no excess of fluid. The lungs are retracted after removal of the sternum. The pericardial cavity is normal.

The Lungs.—Left Lung.—In general the surface is smooth and of a mottled gray and black color. The pleura over the external surface of the lower lobe, near the posterior border, is dull and in places covered with a thin layer of fibrin. About the middle of this surface is a round projecting area about 3 cm. in diameter; its center is of a conspicuous bright-yellow color from which extend outward irregular anastomosing lines of the same color (distended lymphatics). About the yellow center is a wide area of a deep red color. On section, the tissue below the yellow and red surfaces is firm and consolidated. The yellow central portion is quite irregular in shape; at one point it has softened and there is a small cavity containing thin yellow fluid.

Near the basal edge of the lung are two smaller lesions similar in char-

acter to that just described, the larger about 1 cm. across; in neither has the bright yellow portion softened. The remainder of the lung is crepitant, dry on section, and slightly bile-stained. The bronchi contain a moderate amount of tenacious somewhat blood-stained mucus. The bronchial lymph-glands are slightly enlarged, soft and of a red-black color.

Right Lung.—This differs but little from the left. Over the external surface of the lower lobe near the posterior border, the surface is dull and covered in places with a small amount of fibrin. Near the basal edge is a lesion similar to that already described in the left lung. The central bright-yellow area, about 1.5 cm. across, is very irregular in shape and not softened. Irregular lines extend outward from it below the pleura. The lymphatic glands are larger, but resemble those of the left.

Liver.—Weight 4090 grm. The organ is of very large size. In general the surface is smooth, but is studded, particularly over the upper surface of the right lobe, by low boss-like projections whose center is a conspicuous bright-yellow color, about which is a wide irregular zone of very deep-red color. The surface between is of a deep-red tint but not so deep as that about the yellow areas.

The anterior third of the upper surface of the right lobe is in very large part occupied by bright-yellow areas often confluent, between which the surface is of a very deep-red color. On section through the projecting areas, one finds that they represent abscess cavities in the substance of the liver, surrounded by a zone of very deep injection. Though they vary very much in size (see Plate XLII), the usual diameter of the central yellow portion is about 2 cm.; the zone of injection varies greatly and is often continuous between adjacent cavities. A narrow, irregular, somewhat convoluted zone of solid bright opaque-yellow material surrounds a cavity containing thin, slightly turbid fluid. The formation of these abscesses appears to bear a definite relation to the liver lobulation. In the right lobe where abscess formation occupies a large proportion of the substance of the liver, in the hyperaemic tissue abutting upon the abscesses corresponding to the lobules are minute bright-yellow areas of opaque appearance, in the center of which is the section of a small vessel. These minute areas are separated by tissue of red color in which also on careful examination can be seen a vessel cut across or longitudinally. The yellow zone apparently increases in size, and other areas are made up of small rounded areas about 2 or 3 mm. across, composed of somewhat soft yellow tissue and separated by red lines. Finally the central portion of such a collection of small foci softens and the larger abscess cavities with irregular walls are thus apparently formed. In one part an abscess abutted upon and eroded a large branch of the hepatic vein.

In the relatively normal portions of the liver, lobulation is well marked; the tissue is bile-stained and greenish.

Gall-bladder.—The surface is rough, having been in contact with the gauze packing. The wall is somewhat thicker than usual. It contains translucent viscid bile. The gall-ducts are normal.

No abnormality of the hepatic artery or portal vein was discoverable. In the gastro-hepatic omentum are several lymphatic glands about 1 cm. in diameter, soft, succulent, and on section of a dull red color.

Spleen.—Weight 190 grm. Upon the anterior edge an opaque-yellow, slightly raised area, measuring 0.5 cm., occurs. This area on section proved to be an abscess surrounded by a deep-red zone of splenic tissue.

Intestines.—Duodenum and jejunum are normal. The lowermost 100 cm. of the ileum show well-marked injection of the blood-vessels, giving a red color which is most intense in the neighborhood of the ileo-caecal valve. Peyer's patches and solitary follicles are normal. Appendix and colon are normal.

Kidneys.—Combined weight 450 grm. The two organs resemble each other closely. The capsule comes away readily, leaving a smooth yellow-gray surface upon which can be seen minute red areas. The organs are firm in consistence. The cortex is 7 mm. thick, and is pale gray with a yellowish tinge (jaundice). Striae are visible. Malpighian bodies are conspicuous. The mucous membrane of the pelvis is stained yellow, and below, here and there, ecchymoses occur.

The splenic, superior mesenteric and portal veins when cut open show no abnormality.

Microscopical Examination of Hardened Tissues.—Liver.—There is a general dilatation of the capillaries with blood, more especially in and around the central vein, where the liver cells are swollen, hyaline, and without nuclei. There is a general leucocytosis present and occasionally can be seen small clumps of polymorphonuclear leucocytes, some of which show fragmentation or distortion of their nuclei. Large mononuclear cells are to be found here and there, resembling those described by Mallory as being present in the blood in typhoid fever. They evidently are phagocytic, as they contain nuclear protoplasm. In the capillaries certain material is met with at times which stains with haematoxylin and under the immersion lens is found to be composed of bacteria.

The chief lesions present are, however, smaller or larger areas of necrosis. If large, their outlines tend to be lobulated in character, and if small, they have a rounded appearance. These necrotic areas are made

up of three zones. (a) an outer, of varying width, which may occasionally be wanting, made up of polymorphonuclear leucocytes; (b) a middle zone, usually narrow, composed of irregular or round masses of small size, homogeneous and taking the haematoxylin stain, and under very high power these are seen to be made up of bacteria; (c) an inner or central zone consisting of necrotic liver cells and leucocytes whose outlines can only be indistinctly seen, and odd clumps of bacteria. This central zone stains intensely with eosin. Quite often the dead areas of one lobule fuse with those of others leading to the formation of large irregularly-shaped masses of necrotic liver substance.

As regards the anatomical origin of these necroses, it would seem that they arise close to the central vein (rarely within, unless by the lodgement of a bacterial thrombus), and at times in the middle third of a lobule. Now and then areas of necrosis are discovered apart from the presence of bacteria as far as can be seen in the sections.

Nowhere is there any appearance of repair, the process being an acute one.

Lung.—The section contains an abscess similar in character to the larger ones in the liver, namely, wide necrotic areas surrounded by a zone of polymorphonuclear leucocytic infiltration; leucocytes occupy alveoli. The surrounding tissue shows engorgement of blood-vessels and infiltration with red blood corpuscles. A bacterial thrombus is seen at one portion of the periphery of a vein.

Spleen.—The organ contains a similar abscess. The zone of necrosis is very wide and at the periphery there is an abundant blue staining material (bacteria?); the zone of leucocytes is very narrow. The pulp is distended with blood; the small vessels are dilated.

Kidneys.—In many small areas, particularly about the vessels between cortex and pyramid, are areas in which the interstitial tissue is infiltrated with round and plasma cells. The cells of the tubules are large, sharply outlined and granular.

Bacteriological Report of the Autopsy.—At the autopsy, plate cultures were made in plain agar from the blood of the heart, the liver, liver abscesses, spleen, kidney and peritoneal cavity. The plates were placed in the thermostat at 36.5° C., left for 24 hours and examined. All were found to be grossly contaminated by a variety of B. subtilis, due no doubt to imperfect previous sterilization of the plates. Later on, a second series of plates was made in both plain

and hydrocele-fluid agar from the abscesses in the liver, and grown aërobically and in a hydrogen atmosphere at 36.5° C. for 48 hours.

A rabbit was likewise inoculated intravenously with 0.4 cc. of the purulent content of one liver abscess.

The inoculated plates containing both media which were grown aërobically were sterile at the end of 48 hours. The anaërobic plates were not examined until the end of 72 hours when it was found that all the plain agar plates were sterile, while of those containing hydrocele-fluid agar, the first plate alone gave three foci of growth.

Description of the Colonies.—Arising from each of the three small shreds of necrotic tissue could be seen a small zone, about 5 mm. in diameter, of minute white colonies, wholly lying in the depths of the medium.

These zones were found on weak magnification to be made up of more or less oval and round colonies presenting a finely broken-glass appearance. In color they were light brown-yellow, and they were translucent and quite smooth in contour.

Cover-slip Preparations.—Stained preparations showed the colonies to be made up of bacilli, small but variable in length and thickness and having rounded ends. They occur singly, in pairs and in short chains, the latter often resembling streptococci. Treated by Gram's method they become decolorized, and no spores were discovered. From the plates, plain and hydrocele-fluid agar tubes were inoculated and grown in air and in an atmosphere of hydrogen. Colonies developed in the latter tubes kept anaërobically which showed on cover-slips bacilli identical in morphology with those present in the contents of the liver abscess. The bacilli stain readily in carbol-fuchsin and aniline gentian violet.

Bacterioscopic Examination of Contents of Liver and Spleen Abscesses.—Liver.—Considerable numbers of polymorphonuclear and mononuclear leucocytes were present amongst which vast numbers of bacteria were scattered. Apparently one type of bacteria only occurred, which was of a rod form for the most part, although coccus-like forms have been noted. The bacilli varied in length, less so in breadth; some seemed almost the size of typhoid bacilli, whilst the

majority were much more minute. Occasionally one saw forms with slightly swollen ends, which at times stained heavily; more rarely, short filamentous forms were to be seen, and also curved rods. No spores were noticed, although beaded appearances were often seen, resembling B. tuberculosis. Appearances suggestive of branching were noted at rare intervals. The organisms decolorized quite readily by Gram's method of staining and were devoid of spores.

Spleen.—Owing to possible over-heating during fixation, the organisms, which were present in countless numbers, stained poorly and were swollen and slightly refractile. In consequence they were coarser in appearance than those from the liver abscess and more definite and numerous coccus-like forms appeared.

Fresh pus diluted with Dunham's solution was examined as a hanging drop. No independent motility could be observed, but brownian movement was active.

Study of the Bacillus in Pure Culture.—Since it was found that the organism was not able to grow upon any of the ordinary culture media except when human blood or serum were present, placental blood or hydrocele-fluid was added to such media when possible, and the cultural characters of the bacillus studied under those conditions. To avoid the danger of working with mixed cultures from the original plate, the organisms obtained from that source were at once plated both in plain and hydrocele-fluid agar and brought under aërobic and anaërobic conditions for 72 hours at 36.5° C. At the end of that period no growth whatsoever was obtained in the aërobic plates, while multiplications occurred only on the anaërobic hydrocele-fluid agar cultures.

Appearance of Colonies.—To the naked eye all the surface colonies presented the appearances of small colon bacillus colonies, being about 1-2 mm. in diameter. Under the microscope the surface colonies were yellow-brown, coarsely granular, somewhat reticulated, nucleated, rather thick at centers, delicately fringed, translucent and with regular peripheries. The deep colonies were small, oval, round, triangular or irregular in shape, dark-brown in color, granular and translucent. The odor of plates was decidedly and offensively faecal.

Morphology.—The form of the organisms proved to be identical with those obtained from the pus of the abscesses and the original plate culture.

Subcultures were made as before and only those were positive which were made on hydrocele-fluid agar and grown either in a hydrogen atmosphere or according to the method of Buchner. It was noted that growth was equally good and rapid whether incubated in hydrogen or in an atmosphere from which the oxygen had been absorbed by alkaline pyrogallic acid.

Depending upon the density of the agar, variations in appearance of colonies could be obtained. If the agar were thin, it was noted that a deep colony would be surrounded by a swarm of much smaller ones. This effect was less noticeable in surface colonies, but if the agar was denser no such radiating effect took place.

The general cultural characters of the bacillus are set down in Table I.

Further Biological Characters of the Bacillus.—Although a complete study of all the biological characters of the bacillus could not be carried out, certain observations were made which deserve to be mentioned.

Viability.—One plate culture sixteen days old was no longer capable of transplantation, but, as a rule, it was found that most colonies on other plates died out at the end of a week. Tube cultures gave better results under certain conditions. It was found that deep stab cultures lived much longer than streak cultures, that is, when both were left exposed to the air after growing 48 hours in hydrogen. For example, one stab culture was found viable at the end of 42 days, another at 21 days, but of four tubes 16 days old, only one gave positive results on subculture. Streak cultures had usually short lives; of two cultures three days old, one only survived, but another culture four days old still gave subcultures. It would have been interesting had these experiments been made under continuous exposure to hydrogen, as the comparison between deep stab cultures and streak cultures when kept exposed to the air, showed such great difference in length of viability that it seems probable that the oxy-

TABLE I.

Medium.	48 hours at 37° C.	72 hours at 37° C.	6th day (room temp.)
Hydrocele-fluid agar (slant and stab).....	Along line of inoculation are seen a few scattered, elevated, semi-opaque, gray-white, moist, smooth, round, glossy cols., measuring 1-2 mm. in diameter. Stab growth is vigorous, white, granular, and translucent. There are many gas bubbles in the medium and condens. water is cloudy and has a precipitate.	Unaltered.	Unaltered.
Hydrocele-fluid glucose agar (stab).....	Gas formation vigorous. Surface is covered by a heavy condensation-water growth. Stab growth is vigorous, whitish, granular and broken up by bubbles of gas. Some growth is seen in gas bubble clefts.	More gas formation. Growth everywhere more decided.	Most gas is now absorbed, and growth is seen in the gas clefts.
Glycerine agar (plain)...	No growth.	No growth.	No growth.
Potato (streaked with hydrocele-fluid).....	No visible growth. Cover-slip preparations show no increase.	Idem.	Idem.
Bouillon + hydrocele-fluid	Uniformly and faintly cloudy. No scum on surface. An abundant semi-viscid, flocculent, whitish ppt., which on shaking vigorously diffuses with great difficulty, rising up string-like, ¾ the way to surface and then breaking up.	Slightly more cloudy. A froth is seen on surface and gas bubbles are seen rising. Odor faecal.	Less cloudy. The ppt. has increased somewhat.

Litmus milk + hydrocele-fluid	General reaction slightly acid. The cream-ring has a greenish-blue tint. No coagulation, but an appearance as if medium were beginning to clear up.	Slight increase in acidity. No coagulation; same appearance as if peptonizing.	There has occurred a total solution of casein, and medium is now almost transparent and of a dark red-purple color.
Ox blood serum	No growth.	No growth.	No growth.
Gelatine + hydrocele-fluid (shake culture)			No liquefaction. Large numbers of small, round, white, opaque colonies. No gas bubbles seen.
Human blood glucose agar	Gas formation moderate in amount. Growth occurs all way down stab. Odor disagreeably aromatic.		Has been further gas formation as shown by increased number of now empty gas clefts filled with growth. Color of haemoglobin brown.
Bouillon + blood	Faintly and uniformly cloudy. It has a froth on surface and small gas bubbles can be seen rising. There is a white semi-viscid ppt. which diffuses with great difficulty.		Froth has gone. Haemoglobin color lost and replaced by a light dirty brown. Medium more cloudy than before.
Litmus milk + blood	Acid reaction moderate. No coagulation.		Reaction unaltered. No coagulation, no peptonization. Color of haemoglobin has nearly been lost.
Dunham's solution + blood	Very faintly clouded. Has a small amount of white, semi-flocculent, semi-viscid ppt. which diffuses with difficulty.		Still faintly cloudy. The haemaglobin color is still a rich cherry-red. The ppt. has increased in amount.

Motility, negative. Gram, negative. Indol, trace. Spore-formation, not observed in any medium.

gen of the air exerted no feeble bactericidal influence upon the bacilli.

The organism, too, finally developed a tendency towards reproductive enfeeblement, as subculture succeeded subculture, so that it became more and more difficult to induce it to grow, and, at last, it died out at the twenty-third generation.

Thermal Death-Point.—The tests were carried out with a vigorous hydrocele-fluid broth culture three days old, by introducing small quantities into small Sternberg bulbs and enclosing the bulb in a fine-mesh wire box, immersing it completely in water at a given temperature and constantly keeping it moving, at the same time carefully keeping the temperature even. After exposure, the contents of the bulb were expelled into tubes of hydrocele-fluid agar and plated, then incubated for four days in hydrogen at 36.5° C. A control from the broth culture was also made subject to the same technique, excepting, of course, any exposure to heat beyond that required to expel the contents from the bulb. The results were as follows:

Exposure.	Result.
5 minutes at 50° C.	= Positive, 15 colonies.
5 " " 55°	= Negative.
5 " " 60°	= Negative.
5 " " 65°	= Negative.
10 " " 50°	= Negative.
10 " " 55°	= Negative.
Control	= Innumerable colonies.

Hence, a five-minute exposure at 55° C. was regarded as the thermal death-point of the bacillus.

Toxin Production.—An attempt was made to determine whether the organism yielded soluble toxins or not. To this end, 550 cc. of dextrose-free broth, made according to the directions of Theobald Smith, of a reaction of .5 +, were mixed with 185 cc. of hydrocele fluid under aseptic precautions and poured into a Fernbach flask and incubated for 48 hours at 36.5° C. Being found sterile the fluid was now inoculated by emptying into it the whole of a hydrocele-agar

culture of the bacillus obtained from the liver of Rabbit VI. Hydrogen gas was then passed through the flask for three-quarters of an hour and the flask was placed in the thermostat. At the end of 24 hours the growth was tolerably abundant, and upon the fourth day it was very heavy, appearing as a slimy, gray-white layer on the bottom of the flask and clouding moderately and diffusely the supernatant fluid. When thirteen days old the culture was filtered through a Pasteur-Chamberland filter. The filtrate was found upon titrating a portion to have a reaction of 0.85 +, being an increase in acidity of 0.35. A rabbit was given 1 cc. of this filtrate intravenously, and during a period of sixteen days during which it was under my own observation the animal showed no symptoms. Twelve days later the animal was found dead, but owing to the carelessness of an attendant its death was not reported. It would appear from this experiment that no active soluble toxin was produced.

Production of Sulphides.—During the attempt to find a soluble toxin it was thought best to renew the anaërobic condition in the flask by passing fresh hydrogen through the culture. As soon as an opening was made a most foul-smelling outrush of gas occurred, and so powerful was the stench that it could be easily detected throughout the building in which the experiment was being carried on. Suspecting the existence of sulphides, a test was made for their presence in the escaping gas, and a piece of filter paper soaked in a weak aqueous solution of lead acetate was very quickly turned a deep brown color, thus giving positive evidence of their presence. As a control to this a similar piece of paper was held in the stream of hydrogen from the generator for two minutes without showing the least trace of discoloration. When filtered, the filtrate gave no evidence of sulphides in solution when lead acetate paper was soaked in it. The gas collected in fermentation tubes also gave positive tests for sulphides, the atmosphere of the culture having been nitrogen.

Fermentation.—Having previously noted the constant appearance of gas in hydrocele-fluid agar made up with dextrose-free broth, an attempt was made to find out how active this fermentation might be, and what was the composition of the gas. Three fermentation tubes

were filled with hydrocele-bouillon whose reaction was 0.1 +, and inoculated with the organism from an active culture, and incubated four days in a Buchner jar. In each tube 1 cm. of the upper part of the branch was occupied by gas, whose composition when roughly estimated was $\frac{3\,H}{1\,CO}$ although with this was mixed a gaseous sulphide, presumably hydrogen sulphide, as shown by lead acetate paper. What was the fermentescible substance present in the bouillon? As the sugar, it was believed, has been quite removed from the meat extract by previous fermentation with B. coli, it seems not unreasonable to consider that the gas was produced from proteid, especially as it was sulphur-containing. An analogous condition is seen in the abundant gas formation in dextrose-free broth by the action of Bacillus aerogenes capsulatus.

It might be mentioned that egg-cultures could not be obtained, although twice attempted.

Optimum titre of media.—Several tests were made and it was found that the degree of acidity recommended by the Bacteriological Committee of the American Public Health Association, namely, 1.5, was too great and a growth was difficult to obtain in a medium of that reaction, while the organism flourished most vigorously in media between 0.5 and 0.1 acid.

Animal Inoculations.—As previously stated, a rabbit was inoculated intravenously with 0.4 cc. of pus from one of the abscesses in the liver of the human cadaver. This animal died in seventeen hours and showed no naked-eye lesions, and cultures from it were negative; yet the histological examination of the liver showed interesting changes which will be mentioned later on.

With pure cultures of the bacillus further inoculations were carried out upon rabbits, guinea-pigs and mice with results which practically reproduced in many of the animals the hepatic lesions found in the human case, the organism seeming to exercise a selective action upon the liver when introduced into the blood directly or through the abdominal cavity. The results of this series of inoculations are given in the following table:

TABLE II.

Animal.	Date of inoculation.	Material used.	Quantity of same.	How inoculated.	Results.	Cultures (Pos. or neg.)	Length of time taken to kill.
Rabbit I.	Oct. 23rd.	Pus from liver abscess of cadaver.	0.4 cc.	Intravenously.	Died, Oct. 24th.	—	17 hours.
Rabbit II.	Oct. 25th.	Condens.-water of growth from a 3 day-old agar slant, from col. "B." (Gen. 3rd).	0.5 cc.	Intravenously.	Died, Oct. 31st.	+	6 days.
Rabbit III.	Oct. 25th.	Ditto, from col. "A"—3 days old. (Gen. 3rd).	0.3 cc.	Into liver through thoracic wall.	Died, Nov. 6th.	+	12 days.
Rabbit IV.	Nov. 4th.	Condens.-water growth on agar 4 days old, from perit. cav. Rabbit II.	0.25 cc.	Intravenously.	Died, Nov. 10th.	+	6 days.
Rabbit V.	Nov. 4th.	(Ditto).	1 cc.	Subcutaneously.	Died, Dec. 3rd.	—	29 days.
Rabbit VI.	Nov. 11th.	Perit. fluid from Rabbit IV.	1 cc.	Intravenously.	Died, Nov. 16th.	+	5 days.
Rabbit VII.	Nov. 27th.	Condens.-water growth on agar 13 days old, from Rabbit II.	1 cc.	Intravenously.	Died, Dec. 5th.	—	8 days.
Rabbit VIII.	Dec. 5th.	Toxin, 13 days old.	1 cc.	Intravenously.	Died, Jan. 2nd, 1901.	..	28 days.
Rabbit IX.	Dec. 13th.	Bouillon cult. 3 days old.	0.75 cc.	Intravenously.	Died.	—	20 days (?)
Guinea-pig I.	Oct. 25th.	Condens.-water growth on agar 3 days old, from col. "C." (Gen. 3rd.)	0.5 cc.	Intraperitoneally.	Died, Dec. 13th.	—	49 days.
Guinea-pig II.	Nov. 4th.	Condens.-water growth on agar 4 days old, from perit. cav. Rabbit II.	0.5 cc.	Intraperitoneally.	Died, Nov. 8th.	+	4 days.
Guinea-pig III.	Nov. 18th.	Ditto, 2 days old, from liver of Rabbit VI.	0.5 cc.	Intraperitoneally.	Died, Nov. 20th.	+	2 days.
Mouse I.	Oct. 25th.	Ditto, 3 days old, col. "B." (Gen. 3rd).	0.3 cc.	Subcutaneously.	Died, Nov. 6th.	+	11 days.

The gross pathological appearances following upon the inoculation of the animals were so uniform that it seems preferable to give a composite description of the results, rather than to enter into the details found in each animal. Due mention will, of course, be made of any peculiar conditions existing.

After intravenous and intraperitoneal inoculations in rabbits there was rapid emaciation. The chief pathological effects, however, were noted in the peritoneum, liver and spleen.

The peritoneal cavity showed in some cases an acute fibrino-purulent or sero-fibrino-purulent inflammation. The small and great intestines were glued together and attached to the liver, stomach and spleen by fibrinous adhesions, while at times a collection of blood-stained turbid fluid, in which floated small masses of coagulated lymph, appeared in the dependent parts. The vessels of the peritoneum everywhere were deeply injected, but the glossy character of the tissue was rarely lost.

The surfaces of the liver were almost regularly covered by a layer of yellowish-white fibrin which caused the organ to adhere closely to the diaphragm, and the various lobes to one another. On removing this exudate the liver substance was seen to be irregularly studded with yellowish-white, roundish areas which stood out prominently in the dark chocolate coloring of the relatively normal tissue. These areas varied in size from 0.25-0.3 mm. and at times protruded above the general level. On section of the organ it was found that the contents of these foci consisted of a cheesy material. There was no definite zone of inflammation surrounding them, but at times a delicate grayish capsule more resistant to invasion could be seen limiting the larger abscesses. The relatively unaltered liver tissue was softer and much more friable than normal and the lobulations could be made out with difficulty. The gall-bladder was unaffected and usually contained a dark green-colored bile.

Abscesses were absent in the spleen and the organ was enlarged, brown-red in color, soft and friable, and more or less sheathed in exudate which caused it to adhere to the intestines or costal wall.

One rare lesion was that of thrombosis of a splenic vessel causing infarction of the whole spleen, which then was pale red-brown or salmon color, enlarged, firm, and on section, of a homogeneous and dry appearance.

In two instances the adrenal glands were enlarged and pale and oedematous looking. On section, they showed an almost homogeneous structure and were quite moist; no pigmentary layer could be discerned.

In Rabbit No. III the duodenum contained an abscess within its walls about 5 mm. in diameter. Rabbit No. IV showed many small opaque white areas in the walls of the appendix and in the small gut.

In one case (Rabbit No. III) the stomach contained within its walls towards the pyloric end an abscess measuring 0.5 cm. in diameter.

The pleural and pericardial cavities usually contained a very little clear fluid.

The muscle of the heart was pale. In one case only (Rabbit No. II) was any gross lesion noted, and in this there occurred in the walls of the left ventricle and septum ventriculorum a number of small, opaque-white foci measuring 0.25 to 1 mm. in diameter.

In only one animal (Rabbit No. II) were any foci of necrosis found in the brain. This animal showed in both lobes of brain several round opaque-white areas measuring about 2.5 mm. in diameter.

Subcutaneous inoculation of a rabbit was followed in two days by inflammation of an exudative character. Examination on the fourth day showed a tumor beneath the skin averaging 2.5 cm. in extent. The tumor felt tense and was slightly yielding, and the skin over it was reddened. The animal gradually lost flesh but had a good appetite, and the tumor slowly increased in volume and became firm until upon the 18th day it discharged a considerable part of its contents through the skin having become ulcerated. The exuded material was very thick, its color a yellowish-white and without foul odor. Cultures made from this material were negative, and cover-

slips showed nothing amongst the debris which could be said to be organisms. The abscess continued to discharge and more skin over the upper portions sloughed off, leaving a raw surface 3 x 4 cm. which scabbed over, whilst the lower part of the tumor remained prominent and unaltered. The animal grew more emaciated and weak, the scab became detached, and upon the 30th day the animal was found dead. The autopsy showed the existence of a granulating wound corresponding to the slough mentioned. In the axillary region were two round masses the size of hazel-nuts of a somewhat lobulated character, rubber-like consistence and covered by a thin capsule of fibrous tissue. The contents were yellowish-white and of the consistence of stiff putty. There were no signs of peritonitis; the spleen was not enlarged, but was dark red in color and of soft consistence. The liver showed a few small coccidial nodules, its color was slightly darker than usual and its consistence reduced. Everywhere throughout the organ were seen very many small opaque whitish areas, the largest being about 1 mm. in diameter; cover-slips from these areas showed no bacteria.

Cultures from the liver and spleen yielded B. proteus vulgaris and from the caseous subcutaneous nodule and spleen a diplococcus; none of the typical bacilli were found. These organisms found must probably be regarded as secondary invaders, having possibly entered through the skin lesion.

Guinea-pigs are somewhat less susceptible to inoculation with the bacillus. One of these animals lived 49 days and showed, at autopsy, chronic peritoneal adhesions consisting of firm bands of fibrous tissue which united most of the viscera. A second animal showed necrotic lesions of the lungs. The usual lesions met with in rabbits were also encountered in guinea-pigs.

The effect upon mice was imperfectly studied. One mouse was given a subcutaneous injection of the organism and survived 11 days. The only lesion discovered at autopsy was an abscess at the site of inoculation containing yellow-white cheesy material. Strange to say, this animal, of all inoculated in this study, was the only one in

which the bacillus was recovered from the general circulation. The second mouse inoculated succumbed early to a secondary infection.

Pathological Histology.—The microscopic examination of the experimental lesions showed an almost exact reproduction of the appearances given in the description of the material from the human subject; hence this description will be confined to appearances which were not present in the diseased tissues of the original case and to such which in the animals were more severe than were met with in the human tissue.

Liver.—Rabbit No. 1, inoculated intravenously with 0.4 cc. of the pus from an abscess in the liver of the human cadaver; dead in 17 hours. The lesions are early and entirely microscopic. They are confined to the outer or middle zones of many of the lobules and consist of round, densely packed masses of polymorphonuclear leucocytes, among which in rare instances can be found minute masses of bacteria. The liver cells have been utterly destroyed where these foci exist and no trace of them can be found. Contiguous to these or even at some distance from them are irregularly shaped areas of hyaline necrosis of liver cells which take the eosin stain strongly. The nuclei of the hyaline cells may be entirely gone or reduced to bodies faintly staining with haematoxylin, while the endothelial cells of the capillaries show in places karyorrhexis, and may even be obliterated. The hyaline necroses are confined to the outer and middle zones of the lobules. Nothing definite was made out as to their origin. The hyaline areas are free from leucocytic infiltration, excepting that which is general throughout the blood capillaries.

In the case of the subcutaneous inoculation (Rabbit No. V) the lesions are conceivably due to toxin action alone, since no abscesses or bacterial foci could be found anywhere, the disease being limited for a long period to the subcutaneous tissues and axillary glands only. The liver of this animal shows coagulative necrosis in which, in some instances, the cells present a fibroid appearance and in others are shrunken or have disappeared. In the latter conditions there is an accompanying infiltration of phagocytic cells, which themselves

occasionally become necrotic, possibly through toxin activity. Exceptionally, such a focal necrosis is quite replaced by mononuclear leucocytes, in which case the picture recalls that of the so-called lymphoma of the human liver in typhoid fever. Occasionally a definite ingrowth of the connective tissue of Glisson's capsule can be seen between some of the lobules, nipping off and destroying parenchymatous cells.

The most interesting materials for study were derived from Rabbits No. III and No. IV (see Table II). These animals showed the typical abscesses, and in addition an overgrowth of the connective tissue cells of Glisson's capsule which invaded the lobules and ultimately destroyed them, as can be noticed upon tracing the course of events throughout the specimen as a whole. The picture is very like that seen in intralobular cirrhosis in man, except for the fact that the process here is not so general in its distribution, but is confined largely to the neighborhood of the abscesses. The intestinal lesions of Rabbit No. IV were found to be localized in the lymph nodes of the submucosa, which had undergone coagulative necrosis. Bacteria were not found in the intestinal lesions, and it would therefore appear as if the process were due to absorption of toxic products from the acutely inflamed serosa.

The spleen of Rabbit No. II is the seat of anaemic infarction, due to the large and important splenic vessels having become thrombosed. The spleen was also embedded in a thick envelope of newly formed connective tissue, rich in blood-vessels, plasma cells and in small mononuclear lymphocytes. In this connective tissue sheath lies a vein of moderate size containing a well-developed platelet thrombus hanging free in the lumen and completely encircled with a layer of endothelium. To one side of the thrombus, a freshly-formed mass of platelets and leucocytes anchors it to the wall of the vein, doubtless the free-hanging end of the main mass being further down in the vessel.

Rabbit II also presented some features which were not met with either in the human subject or in any of the other experimental

animals, namely, abscess formation in the cardiac muscle and in the cerebral hemispheres. In the heart wall three areas were discovered which showed some slight variation in character. One presented a relatively large central zone of coagulation necrosis with good preservation of the general architecture of the muscle fibers but loss of minute detail of structure. The middle zone consisted of a narrow band which stains deeply in haematoxylin and is made up largely of dense masses of bacteria and disrupted cell nuclei. There are very few leucocytes found in this zone. The outer zone, also narrow, is made up of muscle cells which have undergone hyaline degeneration with loss of their nuclei, and which are under considerable pressure from the growing mass within. Beyond these zones the muscle fibers are relatively widely separated, possibly due to local oedema, while the fixed connective cells are swollen and proliferating in places. The other two foci are probably an earlier stage than the foregoing, and only two zones are present: an outer narrow one of hyaline transformation, and an inner larger one of bacteria, degenerated leucocytes and other cellular elements, but in which no definite muscle structure is to be seen. The small blood and lymph vessels in the neighborhood show distinct evidences of a local leucocytosis, although there is no massing of the cells in close relation to the destructive process. The leucocytes are very sparingly and evenly distributed in the area of local oedema.

The brain lesion is marked by a most extensive coagulation necrosis, in which a few polymorphonuclear leucocytes can be seen in stages of disintegration. Bounding this necrotic focus is a more or less complete zone of dense leucocytic infiltration, consisting chiefly of polynuclear cells, although some mononuclear cells are also present. The lymphatic channels of the pia mater near to the lesion are crowded with white blood-corpuscles, while the blood-vessels of the pia and surrounding cerebral tissue show a greater number of leucocytes than are normally present. The bacteria are found around the periphery of the necrotic zone, for the most part in dense agglomerated masses.

Comparison with Species Already Described.—Aside from the anaërobic bacteria producing tetanus, quarter-evil, botulism, malignant oedema and emphysematous gangrene, with which the organism described in this article clearly has little in common, we shall turn for comparison to the published descriptions of the bacteria concerned in the production of gangrene and fetid abscesses connected with the genito-urinary tract, the intestinal tract, the middle-ear, the cranial cavity, and other portions of the body, to determine whether Bacillus mortiferus is or is not a new species.

Veillon and Zuber have described a considerable number of bacterial species, which were isolated in dextrose agar after the method of Liborius and consisted of bacilli, micrococci and spirilla. A brief description of the various bacillary species will now be given.

Bacillus ramosus (Veillon and Zuber) is a small, slender bacillus which is slightly larger than the bacillus of mouse-septicaemia when found in the smears made from the pus or other exudates; but when examined from cultures the bacillus appears in short chains, forming pairs like the letter V, and in pseudo-filaments, and in forms which show irregular swollen contours, resembling somewhat Bacillus diphtheriae. Moreover, according to Guillemot, the bacillus is often branched. It does not form spores, is non-motile, and it stains irregularly by Gram's method. The optimum temperature of growth is 37° C., and no growth was obtained on gelatin at room temperature. On glucose agar the colonies resemble Streptococcus pyogenes but are finer and more transparent. Sugar-bouillon becomes clouded and exhibits a slight whitish precipitate, with a varying amount of gas production. A fetid odor is given off by all cultures. Growth takes place vigorously in hydrogen and the bacillus lives about one month in cultures. The bacillus is pathogenic for the mouse, rabbit and guinea-pig upon subcutaneous inoculation, following which, well-defined abscesses occur leading to the death of the animals from cachexia in about a week, and after intravenous injection of the rabbit an intoxication only results, no lesions having been found anywhere in the body.

Bacillus fragilis (Veillon and Zuber).—This organism is much smaller than the preceding. It is, as a rule, straight, but occasionally is curved. The ends are rounded, and sometimes, on account of the center taking the stains less deeply than the poles, it resembles a diplococcus. In pus it is often difficult to find because the debris takes the stain along with

the bacillus; it does not stain by Gram's method and no spores are formed, although certain swollen short forms of the organism may be mistaken for them. The organism grows in both agar and gelatin at room temperature and does not liquefy the gelatin. The optimum of growth, however, is that of the thermostat. In sugar broth the growth is relatively abundant and no gas is formed; but in glucose agar a small amount of gas becomes evident, although it is insufficient to break up the jelly. Its odor is fetid. Duration of life in cultures is variable; occasionally it lives for 25 days. The bacillus is pathogenic when inoculated subcutaneously in guinea-pigs, causing abscesses which produce death in about eight days. It is much more pathogenic for rabbits, causing large abscesses which slough through the skin, the animals dying in seven or eight days. Inoculated in the veins, these animals die from a cachexia and the bacilli are not recoverable in cultures.

Bacillus fusiformis (Veillon and Zuber). As seen in pus this bacillus is a fusiform rod with pointed ends, occurring often in pairs. In cultures it preserves largely these characteristics, and in addition elongated, swollen and granular involution forms appear. The bacillus is nonmotile, stains poorly with the ordinary dyes and not at all by Gram's method. It grows at room and body temperatures. Gelatin is not liquefied; very little foul gas is formed; broth is rapidly and strongly clouded; the colonies on agar resemble those of B. coli only they are more transparent. The viability lasts only four or five days. The bacillus is pathogenic for the rabbit and the guinea-pig, causing upon subcutaneous inoculation small abscesses without producing death.

Bacillus furcosus (Veillon and Zuber).—A very small bacillus of peculiar shape, dividing at one end into two branches like the Greek letter γ. In cultures the rods are longer and the ends of the branches become swollen into round or pyriform shaped bodies. The bacillus is nonmotile, stains well with the usual dyes but not by Gram's method, does not grow at room temperature, and requires three or four days to produce visible growth at 37° C. The colonies on the surface of agar resemble those of the pneumococcus. In broth the bacillus forms a fine precipitate and some gas, which has a sour and fetid odor. The organism lives in cultures about twenty days. It is pathogenic for guinea-pigs, producing abscesses upon subcutaneous inoculation, without as a rule causing death. Occasionally they die after the lapse of several weeks from cachexia.

Bacillus serpens (Veillon and Zuber).—Somewhat larger than Bacillus fragilis; it stains with the ordinary dyes, at times the center staining

less readily than the ends, whereby an appearance of spores is given. In fact, spores are not formed. The bacillus decolorizes by Gram's method of staining. It grows in the various culture media, but not invariably, as pairs, pseudo-filaments, and short chains. It has, according to Guillemot, an undulatory or serpentine motion in gelatin cultures, which it renders fluid. The optimum temperature for growth is 37° C., but it slowly multiplies at room temperature. The colonies on the surface of glucose agar resemble those of the pneumococcus. Sugar broth is rapidly clouded and a heavy whitish precipitate forms at the bottom of the tube, leaving the medium clear. In a liquefied gelatin stab culture the deposit of a white flocculent sediment takes place, leaving the fluid clear. Fetid gas is formed in small amount in gelatin and agar. Viability extends to twenty or twenty-five days. The bacillus is less pathogenic for mice, rabbits and guinea-pigs than Bacillus ramosus.

Bacillus funduliformis (Hallé).—This bacillus was found in the vagina in normal and pathological conditions, and in other localities. It is a small, usually curved rod with rounded ends. Cultures often show pleomorphic forms appearing as large swollen rods, curved tortuous branching filaments or ball-like masses. In exudates the bacillus occurs chiefly within the pus cells as masses or as disseminated organisms. It does not stain readily with the ordinary dyes, and decolorizes by Gram's method of staining. Its optimum growth is at 37° C., at which temperature about six days are required for it to become very noticeable. It forms gas in sugar media which has a very fetid odor. When grown in broth in a vacuum it clouds the medium slightly at the end of the third day, but by the tenth day the bouillon becomes almost clear. The odor is quite foul. Rabbits are insusceptible, while subcutaneous inoculation of guinea-pigs gives rise to abscesses which at times become gangrenous; introduced into the peritoneal cavity of guinea-pigs no action is noticeable, and in no case did any of the animals succumb.

Bacillus nebulosis (Hallé).—A small bacillus resembling the bacillus of mouse-septicaemia. Usually straight, it curves occasionally or appears as a rod swollen at the center and tapering at the extremities. It decolorizes by Gram's method, is asporogenous, and shows no involution forms. Growth at 37° C. is slow and no growth is obtained at room temperature. No gas is formed in sugar media. It is inconstant in its pathogenic properties, as it produces abscesses in rabbits and guinea-pigs occasionally. It caused the death of one rabbit.

Bacillus caducus (Hallé).—Incompletely studied. A small bacillus which stains deeply by Gram's method and exhibits no pleomorphism. It survives in cultures three or four days.

Bacillus radiiformis (Rist and Guillemot).—An organism closely related to Bacillus serpens in all its characters; possibly identical.

Bacillus thetoides (Rist and Guillemot).—Closely related to or identical with Bacillus funduliformis.

Unnamed species.—According to Rist, Veillon and Zuber previously described two bacilli under the heads of species "A" and "C." The former is a long, slender, poorly-staining, Gram-negative bacillus. It does not grow in gelatin at room temperature, but grows in bouillon at 37° C. without clouding, forming small flocculi and giving off a fetid odor. It forms no gas in any of the sugar media. Injected into guinea-pigs and rabbits subcutaneously it forms small abscesses but does not kill.

Species "C" is a non-motile bacillus resembling the bacillus of chicken cholera. It appears not to grow in chains, but in sugar agar long and filamentous forms with round or fusiform swellings are noticeable. Bouillon is not clouded, growth occurring in small flocculent masses which collect at the bottom of the tube. All cultures have a fetid odor suggesting decaying cauliflower. The bacillus is destroyed by moist heat in one hour at 53° C. The pathogenic properties of the bacillus are manifested upon subcutaneous inoculation of mice, guinea-pigs and rabbits by the formation of abscesses which have a fetid smell. Mice and guinea-pigs survive the inoculations, but a rabbit succumbed after severe reaction. The bacilli were recovered from the local lesions and, in the rabbit, from the heart's blood.. Rist encountered both these species and thinks that the latter organism may be identical with Bacillus funduliformis of Hallé.

Guillemot partially describes another species which, being as yet incompletely studied, he terms species "A." It is a small rod about the size of Bacillus ramosus but slightly shorter and in cultures is distinguished by forming chains. At the center of the units of a chain one finds a swelling of a round or fusiform shape, which is not of the nature of a spore. The surface colonies on glucose agar are like those of the pneumococcus. Bouillon is not clouded, but forms flocculi which settle at the bottom of the tube.

Cottet, in addition to finding many of the foregoing bacilli, describes three more which he believes to be new. They have not been named by him and are, in consequence, designated by the letters B, C and D. Bacillus "B" is described as being rectilinear, slightly swollen in the middle, tapering at the ends, occurring occasionally in chains of six to twenty units, and rarely in filaments. It is non-motile and decolorizes by Gram's method. It does not grow in gelatin at room temperature.

No mention is made regarding gas production. Cultures survive six weeks. The organism is pathogenic for guinea-pigs, in which upon subcutaneous inoculation it produces abscesses. Bacillus "C" is a regular, slender strepto-bacillus, the chains of which consist of 10 to 15 units. The bacillus is non-motile and stains by Gram's method. A complete study of the bacillus was prevented by its having died early in cultures. Bacillus "D" is a moderately sized, non-motile bacillus with rounded ends, staining poorly with ordinary dyes and decolorizing by Gram's method. It survived in mixed cultures for a long time, but not having been obtained pure, it was not completely investigated.

A comparison of the above series of bacilli with the species described in this paper, as Bacillus mortiferus, leaves no doubt of the fact that the latter organism differs widely from them all. Among the striking differences are the extremely selective character of Bacillus mortiferus, which requires a medium for its growth containing human blood or serum, its more energetic gas production, and its greater and peculiar pathogenic qualities.

Summary.—From a case of hepatic abscess in man a strictly anaërobic bacillus was obtained in pure culture which is not to be identified with any species of pathogenic bacillus hitherto described. This bacillus is closely adapted to the human organism but it is not wholly limited to growth upon or within that organism, since it exhibits a well-marked and strikingly peculiar action upon certain laboratory animals. There would seem to be justification for the erection, in the case of this bacillus, of a new pathogenic species which I propose to call Bacillus mortiferus.

EXPLANATION OF PLATES.

PLATE XLII.

FIG. 1. Drawing of a section of the human liver, showing different stages of the necrotic process. Natural size.

PLATE XLIII.

FIG. 2. Central necrosis of a lobule in the human liver. The dark portions around the periphery of the necrotic area are bacteria. × 75.

PLATE XLIV.

FIG. 3. A portion of the liver of Rabbit No. iii, showing, (a) necrotic material, (b) formation of dense fibrous tissue, (c) lobules more or less involved by invading fibrous tissue arising from Glisson's capsule. × 20.

FIG. 4. Smear preparation of Bacillus Mortiferus.

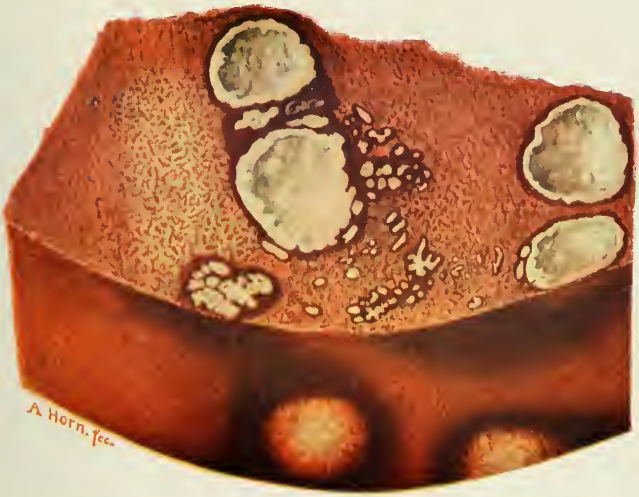


FIG. 1.



FIG. 2.

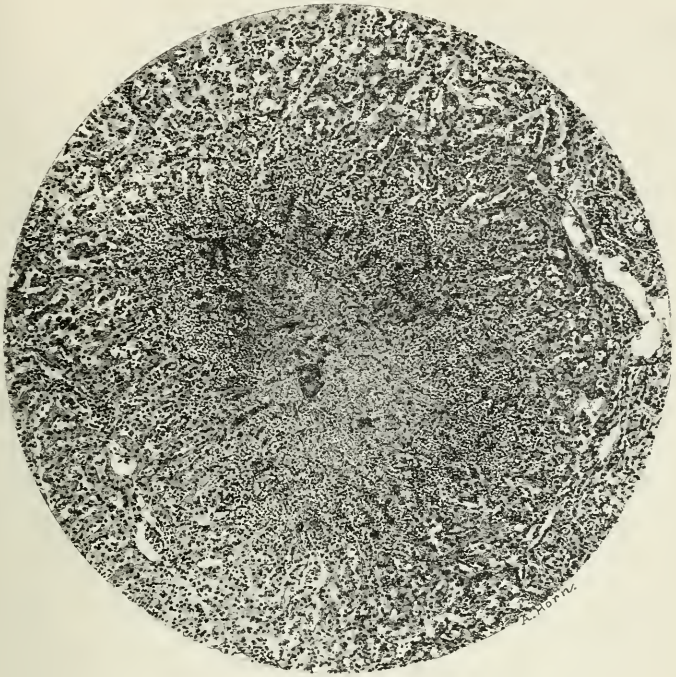


FIG. 3.



FIG. 4.

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Guillemot.—Recherches sur la gangrène pulmonaire. *Thèse de Paris,* 1899.

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NOTE. Since the publication of the preliminary report upon Bacillus mortiferus there have appeared in print two articles upon pathogenic anaërobic bacteria. One by Norris,[1] the other by Albarran and Cottet.[2] The former describes a bacillus obtained from a case of abscess of the liver which bears some resemblance to Bacillus mortiferus, but which differs in morphology, cultural and pathogenic properties. The latter investigated the anaërobic bacteria occurring in abscesses of the genito-urinary tract and added no new species to those already given in this paper.

[1] *Journal of Medical Research*, 1901, I, 97.

[2] *La presse médicale*, 1903, xi, 85.

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